

**A Novel Translocation, t(11;17)(p15;q21), in a Putative Case of Acute Promyelocytic Leukemia. A. Al Saadi, I. Jaiyesimi, J.C. Mattson, William Beaumont Hospital, Royal Oak, MI. A. Al Saadi.** Dept Anatomic Pathology, William Beaumont Hosp, Royal Oak, MI.

Acute promyelogenous leukemia (APL) is a clinical subtype of acute myelogenous leukemia characterized by a coagulopathy, bleeding at presentation, unique morphologic findings, and translocations involving the RARa gene on 17q12-21, and usually the PML gene at 15q21. To date, five partner genes have been identified to participate in the translocation with the RARa gene: PML at 15q21, PMZL at 11q23, NuMA at 11q13, NPM at 5q35, and STAT5b. We report an unusual case of APL in a 48-year-old woman who presented with ecchymoses, a high white count with 55% myeloblasts, anemia, and thrombocytopenia. There was no evidence of a coagulopathy at presentation. The normal bone marrow was replaced by leukemic blasts with reniform nuclei, Auer bodies, but without increased hypergranular promyelocytes. A variant form APL (M3v) was suspected, but standard interphase FISH, using a PML/RARa translocation probe, failed to reveal the classic t(15;17). The patient was started on induction chemotherapy achieving remission after two courses. She was then treated with high-dose Ara C for 4 cycles as consolidation, followed by maintenance therapy with Daunorubicin and Ara C. She did well for 21 months, then relapsed. A repeat bone marrow showed relapsed leukemia. Conventional and FISH cytogenetics, at times of presentation and relapse showed 46,t(11;17)(p15;q21) karyotype with the RARa gene on 17q21 was entirely translocated to 11p15. She was re-induced with Mitoxantrone, Ara C, and Etoposide. Allogenic transplantation is planned after achieving complete remission. This novel translocation, t(11;17)(p15;q21), has not been previously reported in either classic or variant APL. Each of the variant rearrangements reported thus far in putative APL may represent different clinical entities that may require different therapeutic management. Identification and cloning the genes involved in the translocations with the RARa gene may provide valuable information for the understanding of the pathogenesis of the variant APL, and hence for the treatment and management APL with variant translocations.

**The Cancer Chromosome Aberration Project (CCAP): An update.** *T.C. Brown, R. Yonescu, T. Reppert, T. Knutsen, T. Ried, I.R. Kirsch.* Genetics Branch, Center for Cancer Research, Nat'l Cancer Inst, Bethesda, MD.

The Cancer Chromosome Aberration Project (CCAP) was initiated with four goals in mind: (1) to integrate the cytogenetic and physical maps of the human genome, (2) to generate a clone repository of genetically and physically mapped (and in many cases sequenced) DNA BAC clones at a resolution of 1-2 Mb across the human genome, (3) to develop a FISH-mapped BAC clone database to provide a platform for correlation with parallel databases of cancer-associated chromosome aberrations and clinical histopathologic information, and (4) to refine the descriptive nomenclature that defines karyotypic aberrations (insertions, amplifications, deletions, and translocations) in cancer cells. This refinement is designed to supplement and complement conventional descriptions of chromosomes based on banding patterns by integrating and formatting the three data streams of state-of-the-art cytogenetic analyses: spectral karyotyping (SKY), comparative genomic hybridization (CGH), and fluorescent in situ hybridization (FISH). To date, chromosomes 1 (97 clones), 3 (95 clones), 5 (105 clones), 7 (83 clones), 12 (102 clones), 14 (44 clones), 16 (44 clones), 17 (48 clones), 18 (43 clones), 21 (25 clones), 22 (22 clones), and X (60 clones) have been completely analyzed. The data are available at the CCAP website (<http://www.ncbi.nlm.nih.gov/CCAP/>). The results of analysis of the entire set of chromosomes, using ~ 3000 BAC clones, is tentatively set to be available by early fall of this year. Analysis of the 21 mouse chromosomes will follow. CCAP will aid in the elucidation and cloning of chromosomal abnormalities. It contributes to a reagent and data resource that is becoming fundamental to our understanding and management of cancer.

**Numerical, Structural and complex reorganization detected in high resolution (HR) chromosomes in tumor cells from 36 patients with Renal Cell Carcinoma (RCC).** *A.B. Falcon de Vargas<sup>1, 2</sup>, R.E. Vargas Arenas<sup>1, 2</sup>, H. Dvila<sup>2</sup>, M. Uribe<sup>2</sup>, M. Zeeman<sup>2</sup>.* 1) Dept Genetics/Fac Medicine, Hosp Vargas Escuela Vargas UCV, Caracas Dist Fed, Dtto. Capital, Venezuela; 2) Hospital de Clinicas Caracas, Caracas, Distrito Capital, Venezuela.

Abstract: In 36 patients with diagnosis of RCC, 20 males and 16 females with a mean age of 59.94 years (range: 17 to 74), conventional and G, C, Q, NOR banding techniques were carried out in HR chromosomes obtained from tumor cell cultures (TuC) and lymphocytes from peripheral blood (BLy). A total of 644 metaphases in TuC and 1068 in BLy of patients and 1068 metaphases in BLy from 36 healthy controls matched by age and sex were then studied searching for chromosome abnormalities. Results showed numerical and structural changes of chromosomes in patients from RCC group only ( $p < 0.001$ ). Chromosome breakages were found in 60 % of TuC of 30 patients and in 10 % of BLy of 14 patients. In patients with RCC, chromosome abnormalities were found in TuC/BLy as follows: acrocentric segregation in 12/0, polyploidies in 8/0, poly  $>60$  in 24/2, endoreduplications in 12/4, double minute in 12/4, dicentric in 6/0, triradios in 6/0, quadriradios in 4/0. Numerical clonal changes were also found as in TuC/BLy as follows: +7 in 8/2, +7+7 in 4/0, +7+8 in 2/0, +8 in 14/0, +10 in 10/4, +11 in 2/0, +13 in 2/0 and 3 in 2/0, and -8 in 2/0, -10 in 4/0; -X in 4/0 and Y in 8/6. A t (3;8) (3p14-8q24) was found in TuC of two patients. We conclude that clonal numeric and structural chromosome changes are usually present in a high proportion of TuC from RCC and in lower proportion of BLy from these patients. Some of these chromosome abnormalities should be considered nonspecific, since could be seen in other conditions such as different types of cancer, hematological disorders and virus infection, and also induced by mutagenic agents. Further studies are required to determine the clinical value of these changes from the prognostic and therapeutic viewpoint. Key Words: Cancer cytogenetics. Cytogenetics. Chromosomal abnormalities. Genetics. Renal Cell Carcinoma.

**Rapid detection of minimal regions of genomic gain or loss in tumors in order to identify candidate oncogenes and tumor suppressor genes.** *B.L. Gallie<sup>1, 3</sup>, D. Chen<sup>1, 2</sup>, S. Richter<sup>3</sup>, C. Lee<sup>1</sup>, M. Harmadayan<sup>1</sup>, J. Squire<sup>1</sup>.* 1) Princess Margaret Hosp, Toronto, Ontario, Canada; 2) Department of Ophthalmology, 1st Affiliated Hospital, West China University of Medical Sciences, Chengdu, China; 3) Solutions by Sequence, Inc, Toronto Western Hospital, Toronto, Ontario, Canada.

Tumor suppressor genes and oncogenes that are important in the etiology and pathogenesis of particular cancers have been identified by localization of subchromosomal regions of gain or loss in tumors. We have utilized *in silico* methods and quantitative PCR to rapidly delineate the minimal genomic regions likely to contain tumor suppressor genes or oncogenes important in retinoblastoma. Our initial cytogenetic studies using limited numbers of retinoblastoma tumors implicated gains associated with regions of chromosome arms 1q and 6p and losses at 16q. A more comprehensive survey of retinoblastomas using comparative genomic hybridization (CGH) identified consensus regions of gain at 1q31 and 6p22 and loss at 16q22 with a resolution of 20 to 30 Mb. We have developed and validated quantitative multiplex PCR (QM-PCR) to efficiently identify RB1 copy number changes in patient samples. To narrow the consensus regions of gain and loss, we applied QM-PCR to Sequence Tagged Sites (STS) spanning the regions defined by CGH. For the 6p subchromosomal region, CGH narrowed genomic gain to 20 Mb at chromosome 6p22 on 50 retinoblastoma. QM-PCR rapidly reduced the minimal region of gain to 0.5 Mb containing three unigene clusters. A functional relationship between these novel genes and retinoblastoma oncogenesis is being sought. For the chromosomal region 16q22, loss of heterozygosity analysis defined two small regions of overlap at 16q22.1 and 16q23.2 but sample sizes have been limited by the need for matched normal and tumor DNA to define heterozygous loci. We are presently using QM-PCR with STS spanning 16q22 to search large numbers of retinoblastoma tumor DNA samples for minimal regions of deletion, indicative of a tumor suppressor gene. This study demonstrates that the resolving power of QM-PCR and CGH can in combination identify small subsets of the genome containing only a few candidate oncogenes and tumor suppressor genes.



**CGH analysis of colorectal carcinoma.** *S. Cheng.* Dept Anatomy, Chang Gung Univ, Col Medicine, Kweishan, Taiwan.

Tumor has been ranked number one killer among all the diseases in Taiwan. Among all the tumors colorectal carcinoma ranked number three in men and women. This study intends to overview the changes of chromosomal structure of the carcinoma cells. The specimen, both normal and tumor tissues of patients, were collected from the Chang Gung Memorial Hospital at Linkou. The normal and tumor DNAs were extracted and labeled with digoxigenin-16-dUTP and biotin-11-dUTP, respectively. The comparative genomic hybridization was performed on the normal metaphase chromosome spreads. The results were scored on 10 metaphase spreads for each case with the cooled-CCD camera on a fluorescent microscope. The analyses were completed by Quips software (Vysis, Inc.). The main goal is trying to identify the correlation between the metastasis and the common gain or loss of chromosomes in the cells from tumor tissues. The results are from 7 cases with Dukes stages C2 (5 cases) and D (2 cases). All cases are with ulcerative type of tumor tissue. It indicates that the common gains of these case are at chromosome 5 in three cases, at ch 8 in three cases, at ch 11 in three cases, at ch 18 in three cases, and at ch 22 in three cases.

**Sister chromatid exchange (SCE) as a possible indicator of genomic instability associated with ovarian cancer.**

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The transformation of a normal cell, such as an ovarian epithelial cell, to a malignant tumor cell is considered a multistep process that proceeds through an accumulation of mutations in certain genes over the course of many years. It has been proposed that inactivation of DNA repair mechanisms (base excision repair, nucleotide excision repair, mismatch repair, homologous recombination) leads to genomic instability and increased mutation rates, thereby facilitating tumor formation. The proliferation of ovarian epithelial cells following ovulation may involve errors in DNA replication resulting in mutations that may lead to cells with a proliferative advantage. Alternatively, mutations could be caused by environmental agents that reach the ovary through the genital tract. Agents that trigger base mismatch repair and excision repair are efficient inducers of sister chromatid exchange (SCE), suggesting a role for SCE in these pathways in addition to the SCE-related homologous recombination double strand break repair pathway. Using EBV-transformed lymphoblastoid cell lines established from individuals in the NCI-supported Family Registry for Ovarian Cancer and the Gilda Radner Familial Ovarian Cancer Registry, we examined SCE levels in affected individuals and unaffected female relatives. With over 100 paired cell lines available for analysis, preliminary results show an increase in SCE levels in 5/7 probands when compared to unaffected relatives. Mitomycin C challenged cells from these individuals also showed larger increases in SCE levels. Multivariate relationships will be explored between SCE findings and registry database epidemiologic factors as histology, age at diagnosis, race, family history of breast and ovarian cancer, BRCA1 status and polymorphisms in genes involved in DNA repair. [Supported in part by RPCI Alliance Foundation and CA 16056].

**Characterization of complex chromosome aberrations in lymphomas using multi-color fluorescence in situ**

**hybridization.** *J.S. Flores*<sup>1</sup>, *K.C. Kim*<sup>2</sup>, *J. Johnson*<sup>2</sup>, *J. Van Brunt*<sup>2</sup>, *M.M. Li*<sup>2, 3</sup>. 1) Diagnostic Genetic Sciences, Univ of Connecticut, Storrs, CT; 2) Hayward Genetics Center, Tulane Univ Sch of Medicine, New Orleans, LA; 3) Dept of Pediatrics, Tulane Univ Sch of Medicine, New Orleans, LA.

Multi-color fluorescence in situ hybridization (M-FISH) combined with conventional cytogenetic analysis provides a powerful tool for characterizing complex cytogenetic anomalies. Using this approach, we have clarified two lymphoma cases with complex chromosomal aberrations. M. P. was a 53-year-old male suspected to have lymphoma. Cytogenetic studies revealed a karyotype of 42,X,+X,-Y,der(2)t(1;2)(q23;q35),del(8)(q23),t(8;14)(q24;q32),-9,-10,-13,-14,-15,add(16)(q?24),del(17)(p11.2),-18,-19,+mar1,+mar2,+mar3[17]/46,XY[9]. Multi-color FISH confirmed the der(2), t(8;14) and del(17) and eliminated the del(8). It also demonstrated that the add(16) was a der(16)t(15;16)(q24;q24), and that the three markers were der(13;14)(q10;q10)t(8;14)(q24;q32), der(18)dup(18)t(2;18)(18pter@18q23::18q23@18q11.2::2q33) and der(19)t(2 or 10 or 11;19)(v;p13.3)t(13;19)(q14;q13.4). Jumping translocations have been reported in different lymphomas, to our knowledge, this is the first case with a jumping translocation involving chromosome 19 and different donor chromosomes. These results indicate a diagnosis of Burkitts lymphoma. H. S. was a 79 year-old-male referred for chromosomal study because of thrombocytopenia and blasts in peripheral blood. Cytogenetic studies showed a mosaic karyotype: 48,XY,+X,-6,add(7)(q32),+7,der(11)t(11;12)(q23;q13),+11,t(14;18)(q32;q21),der(16)t(16;?),+20,+mar[6]/46,XY[14]. With M-FISH, we were able to confirm the t(14;18) and clarify that the add(7) was der(7)t(6;7)(q23;q32), the der(16) was derived from a t(16;18)(q21;q21) and the marker was a deleted chromosome 11. M-FISH also detected a dup(12)(q13q24.1), which was erroneously identified as a der(11)t(11;12). These results are strongly associated with NHL, especially follicular lymphoma. Our experience in characterizing complex karyotypes in lymphomas demonstrates that multi-color FISH and conventional cytogenetic methods can complement each other and provide valuable information about genetic alteration in lymphomas.

**Genetic alterations in primary breast tumors and their corresponding sentinel lymph node metastasis. L.R.**

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Accumulation of genetic and epigenetic changes is believed to play a pivotal role in each step of tumor development and progression. Studies comparing genomic changes in metastatic tumors with those found in the corresponding primary tumors have revealed differences in the genetic alterations between these lesions, suggesting that specific events may be associated with metastasis or specific clones in the tumor undergo metastasis preferentially. In order to study this process in breast cancer, we are evaluating a group of 10 primary tumors and their matching sentinel lymph nodes metastasis using comparative genomic hybridization. This approach allows us to detect gross chromosomal aberrations present in each tumor and its corresponding node metastasis, and to identify chromosomal alterations common to both the primary and metastatic lesions. Our initial findings show that the most frequent alterations common to the tumors and metastatic lesions analyzed so far include gains of chromosomes 8q, 11q and 20q and losses of 16q and 18q. These findings should be further confirmed using a larger group of tumors. These data will lead to the development of a panel of prognostic markers by selecting FISH probes targeting specific chromosomal regions that are commonly altered in both the primary tumors and their node metastasis.

**Detection of complex chromosomal alterations and gene amplification in Wilms tumors using SKY and array-based CGH.** *M. Goldstein, A. Bar-Shira, H. Rennert, R. Shomrat, C. Legum, Y. Yaron, A. Orr-Urtreger.* Genetic Institute, Tel Aviv Sourasky Medical Cent, Tel Aviv, Israel.

In Wilms tumor (WT) aberrant karyotypes are frequently observed. The most common structural changes reported are at 16q, 11p13, 11p15, 1p, 1q and 7p. G-bands cannot always identify the origin of some chromosomal abnormalities such as markers and detect subtle DNA changes. We used a combination of conventional cytogenetic painting, 24-color spectral karyotyping (SKY) analysis and array-based CGH to characterize complex aberrations in more detail in WTs derived from 3 patients. Case 1 was a 3YO-girl with hemihypertrophy with unilateral WT and nephroblastoma in the second kidney. G-bands analysis revealed a pseudo-diploid karyotype and  $i(7)(q10)$  in 35% of the WT and in 3% of the nephroblastoma cells, respectively, in agreement with the pathologic-exam based diagnosis. Case 2 was a 9YO-boy with recurrent unilateral WT and lung metastasis that demonstrated a hypodiploid karyotype with  $i(1)(q10)$  and  $del(11p)$  in both tumor types. SKY analysis confirmed the primary origin of the lung tumor as WT. Case 3 was a 5YO-girl with metachronous WT. SKY analysis demonstrated a hyperdiploid karyotype in the first WT, and a hypodiploid karyotype in the other kidney-derived tumor. Both WT types displayed complex chromosomal changes involving chromosome 1, while monosomy 7p was observed only in the first WT. Further array-based CGH analysis for simultaneous detection of 59 oncogenes (AmpliOncI Microarray, Vysis) commonly amplified in cancer, revealed a single 3-fold amplification of the multidrug resistance protein 1 (MRP1) gene on 16p13 in patient 3, restricted only to the second tumor. Follow-up by gene expression analysis (HU95A microarray, Affymetrix) showed a 3-fold increase in MRP1 expression level when compared to the first WT and to kidney from a normal control. Increased expression of MRP1 gene was found to be associated with resistance of human tumor cells to cytotoxic drugs and thus, can result in inadequate response to treatment, and adverse prognosis. Combination of SKY and array-based CGH is powerful in characterization of complex rearrangements and detection of novel amplifications.

**Genomic Imbalances in Ovarian Borderline Serous and Mucinous Tumors.** *V. Khanna*<sup>1</sup>, *J. Hu*<sup>1,2,3</sup>, *M.W. Jones*<sup>4</sup>, *U. Surti*<sup>1,2,3,4</sup>. 1) Pittsburgh Cytogenetics Laboratory, University of Pittsburgh Center for Human Genetics and Integrative Biology, UPMC Magee-Womens Hospital, Pittsburgh, PA; 2) Magee-Womens Hospital Research Institute, Pittsburgh, PA; 3) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 4) Department of Pathology, UPMC Magee-Womens Hospital, Pittsburgh, PA.

We analyzed 25 ovarian borderline tumors (13 serous and 12 mucinous tumors) by Comparative Genomic Hybridization (CGH). Genomic imbalance was detected in 85% of serous tumors and 75% of mucinous tumors. Different patterns of the genomic alterations were identified between serous and mucinous tumors. Gain of X-chromosome was common in both serous (30%) and mucinous (42%) tumors. However, gain of chromosome 8 was detected in 38% of serous and mixed sero-mucinous tumors, but not in any pure mucinous tumors. Gain of chromosome 12 was found in 23% of borderline serous tumors, but only in 8% of mucinous tumors. Loss of 1p was found in 23% of serous tumors and in 17% of mucinous tumors. According to the present and previous studies, gain of chromosome 8 is the most common abnormality in borderline serous tumors. Gain of the same chromosome is also common in high grade and advanced stage carcinomas, but uncommon in low grade, low stage carcinomas. In addition gain of X chromosome is common in borderline tumors, while loss of X chromosome is dominant in invasive carcinomas. These findings did not support the multi-step progression theory from borderline tumor to high grade, advanced stage carcinoma, but indicates that the borderline ovarian tumor is a distinct entity. Genes of chromosome 8 may be critical for the development and the differentiation of borderline serous tumors.

**Identification of novel chromosome translocations in human thyroid carcinomas.** *W.O. Lui<sup>1</sup>, A. Svensson<sup>1, 2</sup>, A. Höög<sup>3</sup>, L.O. Farnebo<sup>2</sup>, C. Larsson<sup>1</sup>.* 1) Dept of Molecular Medicine, Karolinska Hospital, Stockholm, Sweden; 2) Dept of Surgery, Karolinska Hospital, Stockholm, Sweden; 3) Dept of Pathology, Karolinska Hospital, Stockholm, Sweden.

Cytogenetic characterization is still significantly important to identify fusion genes. A number of fusion genes are commonly observed in leukemias, lymphomas and sarcomas. In solid tumors, fusion genes have been identified in papillary thyroid carcinomas (PTC), and recently, in follicular thyroid carcinomas (FTC).

The present report describes two cases of thyroid neoplasm with novel chromosome translocations, which were identified by both G-banding and spectral karyotyping. The first case displayed a balanced translocation  $t(3;15)(q11-12;q25)$  in a papillary thyroid carcinoma. The second case demonstrated  $der(2)t(2;10)(q13;q11.2)$ ,  $der(5)t(2;5)(q13;p15.3)$ , and loss of a chromosome 22 in a follicular carcinoma of the right thyroid lobe. Interestingly, the latter case also had a papillary thyroid carcinoma in the left lobe. Unfortunately, the papillary tumor was not available for cell culturing. However, we compared the alterations between these two tumors by comparative genomic hybridization. Both of them showed loss of chromosome 22, but the follicular tumor also displayed loss of chromosome 10p.

In PTC, fusion genes often consist of one of two alternative tyrosine kinase receptor genes, RET and NTRK1. In the case of  $t(3;15)$ , NTRK3, which is mapped at 15q25, and is a member of the TRK family of tyrosine protein kinase genes, could be a putative candidate involving another new novel fusion gene. In the case of  $t(2;10)$  or  $t(2;5)$ , PAX8, which resides at 2q13, had been shown to play an important role in follicular cells differentiation. Recently, a novel fusion gene involves PAX8 and PPAR $\gamma$  was identified in a subset of FTCs. This may strongly suggest that PAX8 could be involved in a putative fusion event with another fusion partner. These findings provide starting points for the exact characterization of molecular events in thyroid neoplasm.

**Banded chromosomes vs Fluorescence In Situ Hybridization (FISH) in the diagnosis of Mantle cell lymphoma.**

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Mantle cell lymphoma (MCL) is a subtype of B cell non-Hodgkin's lymphoma (NHL) characterized by distinct clinico-pathologic features. The cells express pan-B-cell markers, surface immunoglobulin, CD5, and CD43 but typically lacking CD23 and CD10. MCL is associated with t(11;14)(q23;q32) which juxtaposes IgH gene to BCL-1 gene (CCND1, PRAD1) resulting in over-expression of cyclin D1. MCL shares many morphologic and phenotypic features with other NHL. However, it is essential to distinguish MCL from other lymphomas because MCL has an aggressive clinical course and carries poor prognosis. We present three cases with presumptive evidence of MCL that were sent for cytogenetic evaluation. Chromosome analysis was performed on short-term bone marrow aspirate cultures (cases 1,2) and on unstimulated peripheral blood cultures (case 3). The harvests from two cases yielded a normal karyotype while the third case showed the composite karyotype; 46,XY,t(1;22)(p13;q13),-3,del(10)(q22), add(15)(q22), add(17)(p13). The classic t(11;14)(q13;q32) for MCL was not observed in any case. Furthermore, we evaluated the specimens by FISH using the dual color LSI IgH/CCND1 DNA probe (Vysis Inc.). Fusion signals were observed in 65% and 85% of interphase nuclei in case1 and 2 respectively, while the metaphases from both cases revealed a normal pattern. All abnormal metaphases as well as 57% of interphase cells from case 3 displayed a fusion signal that was located on 14q32. Thus, FISH established with certainty the diagnosis of MCL by showing the IgH-CCND1 genes fusion. These findings indicate that the sensitivity of FISH is superior to that of conventional cytogenetics in detecting t(11;14)(q13;q32) associated with MCL.



**The NCI and NCBI SKY/CGH Interactive Online Database.** *T. Knutsen<sup>1</sup>, M. Augustus<sup>1</sup>, V. Gobu<sup>2</sup>, H. Padilla-Nash<sup>1</sup>, E. Schröck<sup>1</sup>, C. Shenmen<sup>2</sup>, J. Plotkin<sup>2</sup>, S. Greenhut<sup>1</sup>, J. Kriebel<sup>1</sup>, K. Sirotkin<sup>2</sup>, T. Ried<sup>1</sup>.* 1) Genetics Branch, National Cancer Institute, NIH, Bethesda, MD; 2) National Center for Biotechnology Information, NIH, Bethesda, MD.

Spectral Karyotyping (SKY) and Comparative Genomic Hybridization (CGH) are complimentary fluorescent molecular cytogenetic techniques. SKY permits the simultaneous visualization of all human or mouse chromosomes in a different color, facilitating the identification of chromosomal aberrations. CGH utilizes the hybridization of differentially labeled tumor and reference DNA to generate a map of DNA copy number changes in tumor genomes. The NCI/NCBI SKY/CGH interactive online database permits the input of clinical and research SKY and CGH data, both human and mouse, directly from the cytogenetic community and the resulting information is available for public viewing in a searchable format. In the SKY database, the submitter enters the karyotype and then describes each abnormal chromosome by typing in the beginning and ending bands of each different segment, starting from the top. The computer then builds a full ideogram, with each chromosome displayed in its unique SKY classification color, with band overlay. Each breakpoint is automatically linked by a button marked FISH to the Map View database, which provides the genes at that site and available FISH clones for that breakpoint. This allows for a seamless integration of cytogenetic aberrations with the sequence of the human genome. Detailed clinical and research information is entered for each case. The CGH database displays gains, losses, and amplification of chromosomes and chromosome segments. CGH data is entered manually, or automatically from CGH software programs. In the manual format, the submitter enters the start band and stop band for each chromosome gain or loss, and the computer program displays the final ideogram with vertical bars to the left or right indicating loss or gain, respectively. This database offers the biomedical community the opportunity to participate directly in the development of data critical to the search for new oncogenes and tumor suppressor genes, and other genetic changes which lie at the root of all cancers.

**HER-2/neu FISH on breast cancer touch imprints of core needle biopsies.** *S. Kaneko*<sup>1,2</sup>, *S.R. Young*<sup>2</sup>, *T. Gerasimova*<sup>2</sup>, *W.M. Butler*<sup>3</sup>, *T.E. Cupples*<sup>4</sup>, *G.R. Greene*<sup>4</sup>, *P.L. Guerry*<sup>5</sup>. 1) Dept OB/GYN, Nagoya City Univ, Japan; 2) Dept OB/GYN, Univ South Carolina; 3) Dept Med, Palmetto Health Alliance; 4) Dept Radiol, PHA; 5) Dept Path, PHA; Columbia, SC.

The HER-2/neu status has been used in breast carcinoma as a prognostic marker and to predict the response to chemotherapy and hormonal therapy and to select patients for HERCEPTIN treatment. Since immunohistochemistry (IHC) is thought to be less reliable, HER2/neu testing with fluorescence in situ hybridization (FISH) is preferred. The analysis of HER-2/neu is usually performed on formalin-fixed, paraffin-embedded tissue sections obtained from surgery. The use of paraffin sections is very time consuming and labor intensive. The objectives of this study were to 1) develop a simple and quick FISH protocol using HER-2/neu and #17 centromeric probes on touch imprints of core needle biopsies, eliminating the need to deparaffinize and pretreat the samples; 2) make the HER-2/neu FISH information available at the multidisciplinary pretreatment tumor conference prior to surgery. A total of 41 core samples were obtained from image guided core needle biopsy. These samples included 26 carcinomas and 15 benign/normal tissues. One carcinoma and one benign sample could not be examined by FISH because of insufficient cells. Three of 25 carcinoma cases were amplified for HER-2/neu. One of three was IHC 3+, one 2+ and one not yet studied. No samples were IHC 3+ and non-amplified. One IHC 2+ was non-amplified. None of the benign/normal samples showed amplification. Only recently has the molecular status of a tumor, such as the presence of a BRCA1/2 mutation, been used to influence surgical decision-making. It is expected that in the near future, it will become more important in the clinical management to take into account the HER-2/neu and other molecular marker status before surgery when the overall treatment plan is being developed. We conclude that HER-2/neu gene analysis by FISH on breast touch imprints is easily done and is a useful and reliable technique.

**In vitro analysis of chromosomal instability in a colon cancer cell line.** *J. Lavoie*<sup>1</sup>, *E. Przybytkowski*<sup>2</sup>, *E. Chudoba*<sup>3</sup>, *R. Drouin*<sup>1</sup>, *M. Basik*<sup>2</sup>. 1) Medical Biol Department, Hosp St Francois d'Assise, Laval Univ, Quebec, PQ., Canada; 2) Surgery Department, Hosp Notre-Dame, Montreal, PQ., Canada; 3) MetaSystems, Altlussheim, Germany.

Colon cancers are characterized by two types of genomic instability: microsatellite instability (MIN) and chromosomal instability (CIN). While the etiology of MIN has been described, little is known about the factors and mechanisms underlying CIN. In order to study CIN in colon cancer, we compared several techniques to measure genomic instability, that is, the rate of appearance of CIN-type genomic alterations over time in culture. The MIN-negative colon cancer cell line, HT-29 was subcloned without selection and two phenotypically distinct subclones were observed: C7 and B6. C7 was phenotypically similar to the parental cell line while B6 was phenotypically similar to another previously described HT-29 subclone, isolated by resistance to methotrexate and 5-fluorouracil. Karyotyping, M-FISH (Multi-fluorescence in-situ hybridization) and inter-simple sequence repeat (Inter-SSR) PCR (a genomic fingerprinting technique) were performed on the original subclones and subclones passaged 15 times (150 generations). Karyotyping revealed fourteen qualitative and quantitative chromosomal anomalies in HT-29 and its subclones: del(X)p11, ins(3;12),del(4)(q),-8,der(8),+11,-13,i(13)(q10),-17,der(19)t(17,19),iso(19q),der(18),-21 and -22. Subclone C7 was identical to the parental line while both were markedly different from B6 (18 different markers). Over the passages, there is a progressive appearance and disappearance of markers (10 changed markers for B6 and 7 different markers over time for C7). Inter-SSR PCR showed similar results to the karyotyping, and allowed the cloning of one of the differences, a loss of heterozygosity of a fragment on chromosome 10. Thus, HT-29 is genomically heterogeneous, although the rate of genomic change seems similar in the different subclones. These findings will form the basis of further studies in the quantification of genomic evolution in colon cancer cells, and allow the future elucidation of the factors underlying the major form of genomic instability in colon cancer.

**Genetic Imbalances in Korean Hepatocellular Carcinoma by Degenerate Oligonucleotide Primed PCR - Comparative Genomic Hybridization.** *G.J. Kim<sup>1, 2</sup>, H.J. Hwang<sup>1</sup>, J.Y. Lee<sup>1</sup>, N.H. Won<sup>3</sup>, S.J. Cho<sup>3</sup>, Y.H. Chun<sup>1</sup>, S.H. park<sup>1</sup>.* 1) Dept. Anatomy, Korea Univ. College of Medicin, Seoul, Korea; 2) Graduate School of Biotechnology, Korea University, seoul, Korea; 3) Dept. Pathology, Korea Univ. College of Medicine, Seoul, Korea.

Hepatocellular carcinoma (HCC) is one of the most frequent gastrointestinal malignant tumors that are prevalent in Southeast Asia. Cytogenetic analysis on HCC has been limited because of poor hepatocyte growth in vitro. Although some genetic alteration related-HCC is now well recognized, the relations between the genetic imbalance and the molecular mechanisms of HCC remain unclear. Recently, technique of degenerate oligonucleotide primed PCR (DOP-PCR) - comparative genomic hybridization (CGH) permits genetic imbalances screening of the entire genome using only small amounts of tumor DNA. Thirty-nine of hepatocellular carcinoma, five of metastatic hepatocellular carcinoma and five of metastatic adenocarcinoma to liver were investigated for chromosomal aberrations by DOP-PCR-CGH on microdissected tissues. The most frequently detected chromosomal gains involved chromosome 1q (55%), 6p (32%), 8q (42%), 17q (87.5%), 20q (75%), and 22q (70%) and the common region of gain involved 7q21-q31 (42.5%) and 12q21-q24 (45%). The most frequently detected loss of chromosomal materials involved chromosomes 4q (67.5%), 8p (30%), and 18q (25%) and the most common region of loss involved 5q14-q23 (35%) and 11q14-qter(25%). The recurrent gain and loss of chromosomal regions identified in this study provide candidate regions that may contain oncogenes or tumor suppressor genes respectively involved in the tumorigenesis of hepatocellular carcinoma.

**Analysis of homogeneous staining regions in cholangiocarcinoma cell lines by chromosome microdissection and FISH.** *Y.H. Kang, G.J. Kim, S.Y. park, H. Kim, S.H. Park.* Dept Anatomy, Korea Univ College Med, Seoul, Korea.

The generation of chromosome region specific painting probe by PCR amplification of microdissected chromosomal DNA has proven extremely useful in identification of chromosomal derivation for marker chromosomes which are indeterminable by routine cytogenetic analysis. The chromosomal derivations of homogeneous staining regions (HSRs) and abnormal band regions (ABRs) in cholangiocarcinoma cell lines, SCK, JCK, and Cho-CK, were analyzed by microdissection and FISH. It was possible to construct the region-specific painting probes for HSRs and ABRs and the probes hybridized specifically to the dissected regions. The painting probes from SCK, Cho-CK, and JCK were hybridized to the chromosome bands 6p21.1-p21.2, 7q11-q22, and 14q13-q24, respectively. Moreover, the fluorescent signals from the amplified chromosomal regions in SCK cells were also easily recognized at almost all the interphase nuclei of SCK cells. These amplified regions were analyzed in cholangiocarcinomas by microdissection and degenerate oligonucleotide-PCR comparative genomic hybridization (CGH). These probe pools for HSRs and ABRs are used to identify the cholangiocarcinoma related genes.

**A comprehensive karyotypic analysis of Korean hepatocellular carcinoma cell lines by cross species color banding and comparative genomic hybridization.** *H.J. Hwang, Y.H. Kang, S.Y. Park, H. Kim, S.H. Park.* Institute of Human Genetics, Dept. Anatomy, Korea Univ. College of Medicine, Seoul, Korea.

Hepatocellular carcinoma (HCC) is one of the most frequent gastrointestinal cancer encountered in the world. Especially incidence rate of HCC in Korea has been increasing. Although some chromosome aberrations appeared in HCC suggest that gene aberrations are involved in tumor formation, origins of many marker chromosomes remain unidentified. The purpose of this study is to establish in detail karyotypes of five HCC cell lines, SNU-368, SNU-449, SNU-398, SNU-182, and SNU-475. The origins of the unidentified marker chromosomes were analyzed by G-banding, cross species color banding (RxFISH), human chromosome-specific painting, and comparative genomic hybridization (CGH). Each cell line had unique modal karyotypic characteristics and showed a variable number of numerical and structural clonal chromosomal aberrations. The origins of the marker chromosomes of these cell lines were confirmed by fluorescence in situ hybridization with constructed painting probes. The gains were commonly detected on chromosomes 7, 20, and 6p. The losses were commonly found 1p36, 4q, 13, 16 and Y. CGH analysis largely confirmed these cytogenetic data. These data suggest that they may be the candidate regions for the isolation of the genes related to HCC.

**Characterization of a Homogeneously Stain Region (HSR) in a new Establishment Hepatocellular carcinoma Cell**

**Line.** *L. Hu*<sup>1</sup>, *J-M. Wen*<sup>2</sup>, *J-F. Huang*<sup>3</sup>, *J.S.T. Sham*<sup>1</sup>, *W-S. Wang*<sup>3</sup>, *M. Zhang*<sup>2</sup>, *D. Xie*<sup>1</sup>, *W-F. Zeng*<sup>2</sup>, *X-Y. Guan*<sup>1</sup>. 1) Clinical Oncology, The University of Hong Kong, Hong Kong, China; 2) Department of Pathology, Sun Yat-sen University of Medical Sciences, Guangzho, China; 3) Department of Sugery, Sun Yat-sen University of Medical Sciences, Guangzhou, China.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and has a very poor prognosis. Recently, we established a HCC cell line from a metastatic tumor. GTG-banding analysis showed that this cell line is hypertriploid (71-78 chromosomes) with a large marker chromosome containing an HSR. A high-copy-number amplification at 11q13 was detected using comparative genomic hybridization. In order to characterize the amplicon, the HSR region was further studied by chromosome microdissection. The dissected DNA was directly PCR-amplified and then mapped to 11q13 by fluorescence in situ hybridization (FISH). DNA copy number of cathepsin F, a tumor invasion and metastasis-related gene at 11q13, has been studied by Southern blot analysis and no DNA amplification was detected. It suggests that the amplified region at 11q13 may harbor another cancer metastasis-related gene. Isolation of amplified transcriptions within the amplicon using microdissected DNA to select cDNA from the HCC cell line by hybrid selection is under the progress.

**Significance of Double Minutes in a Case of Acute Megakaryoblastic Leukemia (M7).** *R. Naeem, R. Bhargava, M. Karamian, R. Hasserjian.* Department of Pathology, Bay State Medical Ctr, Springfield, MA.

**Purpose:** We describe and evaluate the significance of cytogenetic abnormalities (double minutes) in a case of acute megakaryoblastic leukemia (FAB: AML-M7). The patient was a 79-year-old female diagnosed with AML-M7. She received induction chemotherapy with mitoxantrone and etoposide. She received Granulocyte- Colony Stimulating Factor (G-CSF) on the 15th day post induction, but died on the 19th day post induction following an increase in blast number after G-CSF therapy. **Materials and Methods:** Cytogenetic investigations were performed using conventional G-banding technique. Since c-myc oncogene amplification has been previously described in cases of acute myeloid leukemia, fluorescence in situ hybridization (FISH) using c-myc oncogene probe was performed to evaluate that possibility. Flow cytometry was performed to immunophenotype blasts in the peripheral blood. **Results:** Flow cytometry identified a population of blasts which were positive for CD34, CD13, HLA-DR, CD41(gpIIb-IIIa) and CD61(gpIb), consistent with acute megakaryoblastic leukemia (FAB-M7). Cytogenetic studies revealed the following karyotype: 45,XX,-3,del(5)(q22;q33),-7,+mar,dmin. FISH using c-myc specific DNA probe identified only two signals per cell restricted to chromosome 8 on both interphase and metaphase cells. There was no evidence of c-myc oncogene amplification. **Conclusion:** This is the first reported case of AML-M7 to reveal double minutes on cytogenetic studies. c-myc amplification, described in other cases of AML with double minutes was not identified in this case, thus the double minutes may represent amplification of other oncogenes. As has been previously reported, the increase in number of blasts following G-CSF therapy may be related to the cytogenetic finding of double minutes. These findings suggest that growth factors should be used with caution in AML patients whose blasts demonstrate double minutes by conventional cytogenetic studies.



**Identification and analysis of two novel cancer genes located in frequently rearranged chromosomal regions.** *P. Modena, M.A. Testi, T. Mastrangelo, F. Facchinetti, M.T. Radice, S. Pilotti, M.A. Pierotti, G. Sozzi.* Istituto Nazionale Tumori, Milano, Italy.

Sarcomas are rare tumors of mesenchymal origin frequently occurring in youth. Chromosomal translocations generating chimeric genes have been identified in specific tumor subtypes, contributing to the understanding of tumorigenesis and cancer progression. Interestingly, some of these genes are relevant in both haematological and solid tumors, such as FUS-ERG in AML and EWS-ERG in Ewing's tumors. Variant translocations are identified in a minority of cases, whose characterization may disclose new cancer genes. We report the cytogenetic identification and molecular cloning of a t(1;22)(p34;q12) associated with a paracentric inversion of 22q12 invisible by conventional karyotyping in a small round cell sarcoma. By means of cytogenetics and molecular genetics approaches, we cloned the breakpoints both at the genomic and at the cDNA level. All three breakpoints interrupted a gene: EWS on 22q11.2, ZSG on 22q12 and UQCRH on 1p34-35. Three novel fusion transcripts are created: EWS-ZSG and UQCRH-EWS on der22 and ZSG-UQCRH on der1. While EWS is a well-known gene responsible for Ewing's family of tumors, the other two genes have not been previously involved in cancer. ZSG is a putative transcriptional repressor containing POZ/A-T hook/Zn finger domains, and showing similarities to oncogenic proteins PLZF and BCL6. UQCRH encodes hinge protein, a mitochondrial complex III subunit essential for cytochromes c and c1 complex formation. Genomic structure of ZSG and UQCRH genes was delineated, and full length cDNAs of these genes were obtained. Expression studies of the various splicing variants of human and mouse homologs were performed. Fusion and wild type transcripts were cloned into expression vectors to test their transforming activity in in vitro assays. Since frequent LOH is observed in 22q12 and 1p34-35 in solid tumors and the presence of novel cancer genes in these regions has been postulated, we analysed ZSG and UQCRH status in more than 30 cancer cell lines by means of FISH, Spectral Karyotyping, expression studies and mutation detection, with preliminary interesting results.

**CGH analysis of thymic tumors.** *D.J. Heiber<sup>1</sup>, P. Loehrer<sup>2</sup>, G.H. Vance<sup>1</sup>, J.D. Henley<sup>3</sup>*. 1) Dept Medical & Molec Gen, Indiana Univ, Indianapolis, IN; 2) Dept Medicine, Indiana Univ Sch Med, Indianapolis, IN; 3) Dept Path and Lab Medicine, Indiana Univ Sch Med, Indianapolis, IN.

Thymic epithelial tumors include non-invasive thymomas, invasive thymomas and thymic carcinomas and represent the most common anterior mediastinal tumor in adults. Approximately 30-40% of thymomas exhibit malignant behavior and progress or recur with locally advanced, unresectable disease or distant metastases. The malignant behavior of thymic epithelial tumors determines prognosis, but can not always be predicted on histological grounds. To date, there are few reports of genetic alterations in thymic tumors. None have shown a common recurring abnormal numerical or structural pattern by cytogenetic studies. We have evaluated ten thymic tumors by CGH analysis for genome-wide genetic alterations. Specimens included four cortical type thymomas with lymphoid components, two poorly differentiated large cell carcinomas, two metastatic malignant thymomas, one benign thymoma and one thymoma of unknown histology. All ten tumors showed a gain of 10p14-15 which is the location of the BCD1 oncogene, cloned from the lymphocytes of CLL patients, and PFKFB3, found to be required for tumor cell growth. Three of the ten tumors also demonstrated a loss of 19q13.2-13.3, which is the location of the AKT2 gene, whose retrovirus, AKT8, has been isolated from thymoma tumors in mice. The gene ACTN4 also localizes to this chromosomal region and its inactivation has been associated with the metastatic potential of some human cancers. Further analysis of these genes by FISH is ongoing. These studies will potentially identify genes important in the pathogenesis of thymic tumors and may determine markers important for distinguishing indolent from invasive tumors.

**Molecular cytogenetic investigation of Endometrial Polyps by comparative genomic hybridization reveals a novel deletion at 3q27-q28.** *J. Meyer*<sup>1</sup>, *M. Huang*<sup>2</sup>, *N. Kardon*<sup>2</sup>, *K. Hirschhorn*<sup>2</sup>, *T. Mukherjee*<sup>1</sup>, *B. Levy*<sup>2</sup>. 1) Obstetrics and Gynecology, Mount Sinai School of Medicine, New York, NY; 2) Human Genetics and Pediatrics, Mount Sinai School of Medicine, New York, NY.

Endometrial polyps (EP) are common benign tumors found in approximately 25% of the population. They have been associated with menometrorrhagia and infertility. The occurrence of carcinoma in endometrial polyps is less than 0.5%. The molecular events leading to the development and progression of EP remain unclear. To date, cytogenetic studies have been limited to case reports of comparison of G-banded metaphases from cultured cells. Comparative genomic hybridization (CGH), is a molecular cytogenetic technique that permits direct analysis of genomic DNA obtained from the specimen thus obviating the need for cell culture. We used CGH to evaluate DNA sequence copy number changes in 10 EP in order to detect imbalances that are not evident using conventional cytogenetic techniques because of the poor quality of solid tumor karyotypes. In addition, CGH would also detect those cells with imbalances which are lost in culture due to their instability. Seven of the 10 patients underwent hysteroscopy for peri-menopausal bleeding and the remaining 3 patients had EP detected during routine workup for infertility. CGH analysis of EP demonstrated chromosomal imbalances in 4 of 10 (40%) specimens, 3 of which demonstrated an identical deletion at 3q27-q28. A gain at 1q21-q43 and a loss of 7p21 was evident in the other specimen. Of particular interest was the fact that the deletion of 3q27-q28 was also found in surrounding endometrium and only in those patients with infertility. A deletion at 3q27-q28 has not previously been described in endometrial polyps, endometrial hyperplasia or endometrial carcinoma and represents a novel finding. This discovery could reflect a molecular change in the endometrium which predisposes to infertility and/or polyp formation. Identifying and correlating chromosomal abnormalities in EP may provide insight into the etiology and mechanism of this disorder and thus allow for the development of strategies for clinical management and treatment.

**A complex three-chromosome rearrangement with other abnormalities in a case of refractory anemia.** *N.S. Mitter, P.O. Ivbievbiokun, V.L. Hopwood, S. Miao, R.W. Rodgers, K.H. Thompson.* Center for Medical Genetics, Houston, TX.

A hitherto unknown complex rearrangement involving one #5 chromosome and both #8 chromosomes was observed in a 72 year old woman with a significant leukopenia, refractory anemia, thrombocytopenia and severely hypocellular marrow. While 9/22 fully analyzed cells had a normal female chromosome complement of 46,XX, 13/22 cells showed the three-chromosome rearrangement and del(20q), with three of these cells also lacking a #18 chromosome. The complex three-chromosome rearrangement originated from an apparently balanced reciprocal translocation between two homologue #8 chromosomes, followed by an inverted insertion of segment q15q33 from a #5 chromosome in the long arm of one of the two derivative #8 chromosomes. The karyotype was 46,XX,t(8;8;5)(q21.2;q24.1;q15q33),del(20)(q11.2)[10]/idem,-18[3]/46,XX[9]. The long form nomenclature for the complex chromosome rearrangement would be written as t(8;8;5)(8pter->8q21.2::8q24.1->8qter; 8pter->8q24.1::5q33->5q15::8q21.2->8qter; 5pter->5q15::5q33->5qter). A deletion of the long arm of a #20 chromosome, typically seen in many cases of refractory anemia, was also present in all the cells. The complex chromosome rearrangement has not been described as associated with a specific hematologic abnormality. The breakpoint on the insertion recipient #8 chromosome (8q24.1) is the site for myc and pvt1 oncogenes, and has been implicated in many cases of acute lymphocytic leukemia and non-Hodgkin's lymphoma. However, the breakpoints on the #5 chromosome segment, inserted into #8 chromosome, have been involved in many different types of hematologic abnormalities, including myelodysplasia, acute myelogenous leukemia and acute lymphocytic leukemia. The patient responded favorably initially to a treatment with Prednisone. After three months, the patient had become more pancytopenic and a repeat bone marrow showed an 80% cellularity, with 38% blast cells and a histological diagnosis of acute myelogenous leukemia. Based on this observation, the breakpoints on the insertion donor chromosome #5 seem to have been more involved than those on the #8 chromosomes in the leukemic progression in this patient.

**Prognostic implication of chromosome 13 aberrations in B-cell hematopoietic malignancies requires FISH analysis as an upfront screening tool for accurate detection.** *J.L. Murata-Collins, L. Tcheurekdjian, P. Gaytan, M.L. Slovak.* Dept Cytogenetics, City of Hope National Med Ctr, Duarte, CA.

Deletions involving chromosome band 13q14.3 are common in chronic lymphoproliferative disorders (CLPD), including B-cell chronic lymphocytic leukemia (B-CLL) and multiple myeloma (MM); however, their prognostic impact is dissimilar. In B-CLL, patients with 13q14 abnormalities typically have a benign disease course, with overall survival equivalent to age- and sex-matched controls. In contrast, 13q14 abnormalities in MM are associated with a significantly inferior outcome and poor prognosis, suggesting the need for aggressive therapeutic options. Detection of aberrations by standard cytogenetics (SC) in CLPD is hampered by the inability to obtain mitotic malignant B-cells, identifying only a fraction of the chromosome 13 aberrations detected by fluorescence *in situ* hybridization (FISH). In a preliminary FISH study of 43 CLPD samples a dual color, dual loci FISH probe set was created to identify the minimally deleted region in 13q14.3 (BAC 480p3 which includes D13S319), distal to RB1, and 13qter (BAC 19o15), as the control chromosome 13-specific identifier. 8/23 [35%] B-CLL samples showed loss of the 13q14.3 signal, with retention of the control 13qter signal. Discordance in 6/8 samples was due to either a microscopically cryptic deletion or failure to acquire mitotic B-CLL cells. Overall, FISH increased detection of chromosome 13 aberrations by 22% in B-CLL, compared with 13% detection by SC. In 5/20 [25%] MM samples, both 13q14.3 and 13qter signals were missing, consistent with monosomy 13 or a large terminal deletion. Of the 5 abnormal MM samples, SC and FISH results were concordant in 2, single del(13) findings by SC confirmed as clonal in 2, and one normal study suggested failure to culture malignant B-cells. As expected, deletions with breakpoints proximal or distal of BAC 480p3 were negative by FISH, as was the case in 2 B-CLL and 2 MM samples. To assess prognosis in CLPD, chromosome 13 deletions by FISH should be considered an upfront screening tool.

**Evolution of clonal chromosome abnormalities in neuroblastoma studied by comparative genomic hybridization.**

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Loss of chromosome 11q material occurs in approximately 20% of advanced stage neuroblastoma and forms a distinct genetic subtype. Loss of 11q material is strongly associated with loss of distal 3p material and unbalanced gain of 17q material, but is inversely correlated with MYCN amplification and deletion of 1p. The loss and gain of the 11q and 17q regions, respectively, is sometimes the result of an unbalanced reciprocal translocation between the long arms of these chromosomes, while the cytogenetic mechanism of 3p loss is unknown. We have performed CGH analysis on neuroblastomas from 34 different patients in order to further study the clinical behavior and chromosomal abnormalities associated with 11q- tumors. Tumors from 8 of the patients had loss of 11q material. The rearrangements leading to loss of 11q material were often associated with partial gain of genetic material from 11q13 and 11p by complex mechanisms. In one patient (No. 31), CGH, G band cytogenetic and/or FISH analysis could be carried out on neuroblastoma cells from primary tumor, an infiltrated bone marrow sample and a lymph node metastasis. Although gain or loss of genetic material from eleven different chromosomal regions could be detected, partial monosomy 11q and trisomy 17q were the only abnormalities common to tumor cells from all three sites, indicating an early origin during the development of the primary neuroblastoma. Unbalanced gain of 17q and loss of 11q material resulted from an unbalanced t(11;17)(q14;q11.2). Loss of chromosome 3p and gain of 7q was detected at two sites (primary tumor and bone marrow) by CGH and resulted from an unbalanced t(3;7)(p21;q10). We conclude that the t(3;7) is secondary to the t(11;17), and the loss of 3p material is not linked to loss of 11q by a common cytogenetic mechanism.

**Comparison of intra-abdominal leiomyosarcoma and uterine leiomyosarcoma using comparative genomic hybridization analysis** . *W.W.H. Weng<sup>1</sup>, J. Åhlén<sup>2</sup>, W.O. Lui<sup>1</sup>, J. Wejde<sup>3</sup>, C. Larsson<sup>1</sup>*. 1) Department of Molecular Medicine, Karolinska Institute, Stockholm, Sweden; 2) Department of Surgery, Karolinska Hospital, Stockholm, Sweden; 3) Department of Pathology, Karolinska Hospital, Stockholm, Sweden.

Leiomyosarcomas (LMSs) are principally tumors of adult life and are more common in women than men. Although all LMS are histologically similar, they are divided into anatomic groups because of clinical and biological differences. Herein, we used comparative genomic hybridization (CGH) to study the DNA copy number changes of 11 intra-abdominal LMSs (ALMSs) from 8 patients and compared with the CGH result of uterine LMSs (ULMSs) obtained from the literature. CGH demonstrated chromosomal imbalances in 10 of 11 ALMSs. Gains were commonly seen at chromosome 17p(55%), 17q(45%), 5(36%), 7p(36%) and 15(36%) in the ALMSs. These regions of gain are similar to ULMSs, however at a lower frequency. For those tumors exhibiting gain of 17p, high-level amplification was observed in 67%, suggesting that this region may contain an oncogene that involved in LMS tumorigenesis. The most frequently losses detected in the ALMSs were at Xp (73%), Xq (45%) and 4q (45%). However, gain is observed at the X chromosome in ULMSs. Other losses detected at 1p, 9p, 10p, 10q and 13q in the ALMSs are also seen in ULMSs. This shows that intra-abdominal and uterine LMS shared some common genetic alterations that may be involved in the tumorigenesis of this tumor.

**Marker chromosome evolution in oral squamous cell carcinoma (OSCC) cells may occur via breakage-fusion-bridge cycles.** *S. Reshmi-Skarja*<sup>1</sup>, *W.S. Saunders*<sup>2,3,4</sup>, *D.M. Kudla*<sup>1</sup>, *S.M. Gollin*<sup>1,3,4</sup>. 1) Dept. of Human Genetics, University of Pittsburgh GSPH, Pittsburgh, PA; 2) Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA; 3) University of Pittsburgh Oral Cancer Center of Discovery, Pittsburgh, PA; 4) University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Oral squamous cell carcinomas (OSCC) are characterized by near-triploid, highly complex karyotypes. Studies of colon, breast, pancreas, prostate, and oral cancer cell lines reveal that cells within a culture express numerical and structural variations on a background of clonal chromosome aberrations. This indicates the presence of chromosomal instability. Previously, we have presented evidence that gene amplification in OSCC appears to occur through breakage-fusion-bridge (BFB) cycles. Based on our results of sequential trypsin-Giemsa banding and spectral karyotyping (SKY) analysis of OSCC cell lines, we propose that a similar mechanism explains the observation that marker chromosomes within a cell line appear to be related structurally, yet vary (marker chromosome evolution). However, between cell lines, marker chromosomes seem to be unrelated. To test the hypothesis that marker chromosome evolution occurs by BFB cycles, we analyzed at least 8 cells each from two different passages of *TP53* wild-type OSCC cell lines [UPCI:SCC070 (passages 24 and 35), UPCI:SCC122 (passages 19 and 24) and UPCI:SCC136 (passages 36 and 42)]. Each cell line was remarkably stable between the two passages despite expressing numerous shared (identical or evolved) markers and one or two new clonal marker chromosomes. Furthermore, within any cell line analyzed, no two cells had identical karyotypes. These results support the idea that intrinsic structural chromosomal alterations may disrupt chromosome integrity and lead to further chromosomal instability in OSCC. Studies are underway to determine whether the marker chromosomes are present in anaphase bridges. [Supported in part by NIH grants P60DE13059 and P30CA47904.].



**Neurofibromatosis type 2 in a female patient with a balanced de novo X;22 translocation.** *F.L. Raymond<sup>1</sup>, C. Bovie<sup>1</sup>, S. Holden<sup>1</sup>, A. Schroer<sup>2</sup>, D. Trump<sup>1</sup>.* 1) Dept Medical Genetics, Cambridge Inst Medical Res, Cambridge, England; 2) Max-Planck-Institut fur Molekulare Genetik, Ihnestrasse 73, 14195 Berlin, Germany.

Neurofibromatosis type 2 (NF2) is commonly associated with mutations in the gene, merlin. We report the first case of a women with NF2 who carries a de novo balanced reciprocal translocation 46,X,t(X;22)(p11.2;q11.2). We excluded the common mutational mechanisms for NF2 in this patient. A large whole gene deletion was excluded by fluorescent in situ hybridisation using a P1 artificial chromosome dJ76B20, smaller intragenic deletions by Southern blot analysis and point mutations in merlin by DNA sequence analysis of all 17 exons. The NF2 gene is not disrupted at the translocation breakpoint which is located 6 Mb from the 22q11.2 breakpoint. We demonstrate that in tumour tissue in contrast to peripheral blood lymphocytes there is evidence for an aberrant X inactivation pattern and this is likely to be the underlying cause of disease in this individual.

**The origin of marker chromosomes in pediatric and adult ALL elucidated by m-FISH.** *E. Rajcan-Separovic<sup>1</sup>, O. Ludkovski<sup>1</sup>, W. Duey<sup>1</sup>, C. Salski<sup>2</sup>, D. Horsman<sup>2</sup>, V. Lestou<sup>2</sup>.* 1) Cytogenetics Laboratory, BC Children's Hospital, Vancouver, Canada; 2) Cytogenetics Laboratory, BC Cancer Agency, Vancouver, Canada.

Cytogenetic analysis plays an important role in the diagnostic work-up in ALL patients. Karyotypic abnormalities have been reported in 60% of ALL patients, with hyperdiploidy having the best prognostic impact in both pediatric and adult ALL. However, the presence of structurally abnormal chromosomes in a hyperdiploid karyotype has been noticed to have an unfavorable influence on disease outcome. The identification of the composition of structurally abnormal chromosomes in ALL and other types of cancer has been one of the most challenging tasks for cytogeneticists. When the origin of a structurally rearranged chromosome cannot be recognized by standard G-banding, it is classified as a marker chromosome. In ALL, markers are reported in 30% cases and of these, 30% are found in hyperdiploid ALL. Currently, the use of multicolor fluorescence in situ hybridization (m-FISH) provides a valuable aid in elucidating the nature of almost every chromosomal rearrangement, including markers.

We have used m-FISH to identify the origin of marker chromosomes from otherwise structurally normal hyperdiploid karyotypes in six cases of pediatric ALL. In all these cases the marker chromosomes appeared to be of single chromosome origin, resulting most likely from an intrachromosomal rearrangement that prevented their identification by G-banding. On the other hand in 5/6 cases of pediatric and adult ALL with hyperdiploid or pseudodiploid karyotype and multiple structural rearrangements including markers, the markers were of multi-chromosomal origin. Our findings suggest that: 1. in hyperdiploid ALL with no structural rearrangements other than marker chromosomes, the markers are likely to be of single chromosomal origin 2. in hyperdiploid and pseudodiploid ALL with multiple structural chromosomal rearrangements including markers, the markers are more likely to be of complex, multichromosomal origin. Further studies of hyperdiploid ALL by m-FISH will help establish if the complexity of markers, and the karyotype, has an influence on disease development.

**Combined cytogenetic and multicolor-FISH analysis of chromosomal abnormalities in erythroleukemia (AML-M6).** *N.G. Wolf, M. Kochera, S. Schwartz.* Case Western Reserve Univ. and University Hospitals of Cleveland, OH.

Erythroleukemia (EL) is a relatively rare form of acute myelogenous leukemia (AML-M6) characterized by the neoplastic proliferation of erythroblasts and myeloblasts. It is a heterogeneous disease often associated with complex karyotypes and an aggressive clinical course. Analysis of EL using classical cytogenetic techniques (G-banding) can be difficult due to the frequent presence of marker chromosomes and of translocations involving chromosomal material of unknown origin. To more accurately assess EL karyotypes, we complimented G-banding analyses of five cases of EL with multicolor-FISH (M-FISH), a molecular cytogenetic technique that allows simultaneous visualization of each chromosome in a different color. M-FISH analysis allowed the delineation of 1 ring and 2 marker chromosomes, and of unidentified material on 9 derivative chromosomes, thereby indicating that apparently missing material from several of the deleted or missing chromosomes had actually been translocated or rearranged. M-FISH analysis also revealed a hidden translocation, confirmed one suspected structural rearrangement, reclassified two others, and found abnormalities (including a second cell line in one case and subclones in two others) not detected by G-banding. Two very small marker chromosomes and one derivative chromosome required additional single or dual-color FISH analysis for determination of their identity. Other aberrations were confirmed with whole chromosome paint or specific FISH probes. The only consistent abnormality, observed in all 5 cases, was loss of all or part of the long arm of chromosome 5, which has been frequently reported in AML (including M-6). This work demonstrates the importance of M-FISH in the study of EL cytogenetics because M-FISH can (1) delineate marker chromosomes and unidentifiable material not resolvable by standard banding, (2) detect cryptic rearrangements, and (3) locate material previously thought to be deleted or missing. Utilizing this approach to more accurately evaluate the chromosomal abnormalities in EL should prove helpful in resolving subclassifications of AML-M6 and provide useful diagnostic and prognostic information.

**Histological comparisons of testicular germ cell tumors by CGH analysis.** *L.R. Smith<sup>1</sup>, O. Henegariu<sup>2</sup>, V.C. Thurston<sup>1</sup>, G.H. Vance<sup>1</sup>.* 1) Dept Med & Molecular Genetics, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Dept of Genetics, Yale Univ Sch Medicine, New Haven, CT.

Twenty-five fresh testicular germ cell tumors (TGCTs) were collected and identified by specific histological subtype. Whereas conventional cytogenetics is limited to the number and quality of metaphases scored, comparative genomic hybridization (CGH) screens the entire genome for chromosomal imbalances and provides a more thorough and sensitive method by which to detect changes in genome copy number. The goal of this project was to analyze the TGCTs by CGH and to delineate gains/losses of genetic material by specific histological subtype. Seven of 25 tumors analyzed were normal with the remaining 18 tumors (9 mature teratomas, 2 metastatic mature teratomas, 2 yolk sac tumors, 2 seminomas, 1 nonseminoma, 1 choriocarcinoma, and 1 mixed GCT) abnormal by CGH analysis. The amplification of 12p, a known chromosomal abnormality in TGCTs, was present in all the 18 abnormal tumors. Gains of genetic material were also identified to chromosomes 17p, 17q, and Xp; losses were identified to chromosomes 4q, 5q, 6q, and 13q. Further, of the 18 abnormal tumors with amplification of 17p, ten had selective gain of 17p13. Eight of these ten tumors (80%) were teratomas (6 mature teratomas and 2 metastatic mature teratomas) with the remaining two of different histological subtype (1 yolk sac tumor and 1 mixed GCT). Although the amplification of 17p13 detected in this study is not unique to histological subtype, it appears to be novel in the teratomas and may represent a chromosomal region containing genes important for cellular differentiation. Our data also substantiate the sensitivity of CGH over conventional cytogenetics. G-banded karyotypes from five of the 18 abnormal tumors were normal whereas the CGH analysis on these same tumors (and others) revealed previously undetected gains/losses of genomic material. Further, the data revealed a gain of 17p13, prominent in teratomas and not previously reported in the literature.

**Analysis of chromosomal imbalances in Korean non-small cell lung cancer using degenerate oligonucleotide primed - PCR comparative genomic hybridization.** *S.Y. Park<sup>1</sup>, G.J. Kim<sup>1</sup>, Y.H. Kim<sup>2</sup>, H. Kim<sup>1</sup>, S.H. Park<sup>1</sup>.* 1) Inst Human Genetics, Korea Univ College of Medicine, Seoul, Korea; 2) Division of Hemato-oncology, Korea Univ. College of Medicine, Seoul, Korea.

To identify genetic alterations associated with the development and progression of non small cell lung cancer (NSCLC) in Korea, 60 tumors were analyzed using degenerate oligonucleotide primed PCR and comparative genomic hybridization (DOP-PCR-CGH). In 59 cases chromosomal imbalances were found. Several recurrent chromosomal abnormalities were identified in the present study. The most frequently detected chromosomal gains involved chromosomes 3q (43%), 21q (43%), 1q(31%), 5p(29%), 7p(31%), 8q(31%), 17q (31%), 20p (31%), 22q (35%), and Xq (31%). Common regions of gain involved 3q26-qter, 21q13-qter, and 22q13-qter. The most frequently detected chromosomal losses involved chromosomes Yq (38%), 3p13-pter (11%), 4q25-q26 (14%), 4q31 (17%), 8p21-pter (14%), 17p12-pter (11%), and 19p (11%). The recurrent sites of chromosomal gain and loss might be candidate regions for abnormal genes involved in the tumorigenesis of NSCLC.

**A unique insertional translocation in a man who had childhood Wilms tumor, ascertained through an offspring with bilateral retinoblastoma.** *I.E. Teshima<sup>1,6</sup>, M. Krinsky<sup>1</sup>, E. Heon<sup>2,3</sup>, J. Sutherland<sup>2</sup>, B.L. Gallie<sup>3,4</sup>, H.S.L. Chan<sup>5,6</sup>.*  
1) Pediatric Lab Medicine, Hosp for Sick Children; 2) Ophthalmology, Hosp for Sick Children; 3) Ophthalmology, Univ of Toronto; 4) Cancer Informatics, UHN; 5) Pediatrics, Hosp for Sick Children; 6) Pediatrics, Univ of Toronto, Canada.

Retinoblastoma and Wilms tumor are rare embryonic neoplasms caused by the loss/inactivation of both alleles of two different tumor suppressor genes: the RB1 within 13q14 and the WT1 gene within 11p13. Interchromosomal insertional translocations are also rare with an incidence of 1:80,000. We report a unique insertional translocation between chromosomes 11 and 13, present in a man who had Wilms tumor in childhood, and whose child had bilateral retinoblastoma. The 2-year-old girl presented with bilateral leukocoria, glaucoma and diminished vision. She was noted to be mildly dysmorphic from birth and subsequently found to be developmentally delayed. Examination of her eyes under anesthesia detected severe bilateral Reese-Ellsworth Group Vb retinoblastoma. Her otherwise normal father gave a history of unilateral Wilms tumor treated with nephrectomy and chemotherapy at 7 years of age. The family history was negative for other malignancies. The retinoblastoma tumors are presently responding to chemotherapy and focal therapy. Cytogenetic studies of the child's cultured lymphocytes identified a constitutional karyotype 46,XX,del(13)(q14.1q14.3) by GTG banding. The deletion was confirmed by FISH using a probe that spanned exons 3 to 17 of the RB1 gene. Parental cytogenetic investigations revealed that this deletion was a result of an unbalanced segregant from the father who had an insertional translocation 46,XY,ins(11;13)(p13;q14.1q14.3) by GTG banding and FISH. The mother's karyotype was 46,XX. Breakpoint investigations will be done to determine whether position effect or bisection of the WT1 had caused the Wilms tumor in the father. Insertional translocations have been previously described in two families with retinoblastoma and in one with Wilms tumor, but not in a family with both tumors. This insertional translocation poses high risk for the family and has unique issues for genetic counseling.

**Structural rearrangements of *Genesis* in primitive neuroectodermal and testicular germ cell tumors.** *G.H. Vance<sup>1</sup>, T.A. Gobbett<sup>1</sup>, H. Ramsey<sup>2</sup>, L.R. Smith<sup>1</sup>, V.C. Thurston<sup>1</sup>, R.A. Hromas<sup>2</sup>.* 1) Dept Medical & Molec Gen, Indiana Univ Sch Medicine, Indianapolis, IN; 2) Dept Medicine, Indiana Univ Sch Medicine, Indianapolis, IN.

Genes that control transcriptional regulation are primary targets for investigations into the mechanisms of cancer. One family of transcriptional regulators is the winged helix family. We have previously cloned and characterized a novel member of this family, *Genesis*, important in normal and malignant germ cell development<sup>1</sup>. *Genesis* has an open-reading frame of 465 amino acids and is specifically expressed in the embryonic histological subtype of non-seminomatous germ cell tumors (GCT). It has been localized to chromosome 1p31, a region of consistent abnormalities in testicular carcinoma. We present the data from 100 tumors analyzed by dual-colored FISH for numerical abnormalities and structural rearrangements of *Genesis*. Of the 100 tumors, 11 specimens were paraffin-embedded sections from primitive neuroectodermal tumors (PNET) and 89 were cultured surgical specimens from primary and/or secondary GCT of varying histology. FISH probes included a PAC with a 145 kb insert (*Genesis* 1.5 kb) that maps to chromosome 1p31.2-1p32.2 and a control PAC mapping proximal at 1p31.1-1p31.2. Eight tumors (1 PNET and 7 GCT) examined by FISH demonstrated aberrant signal patterns characteristic of structural rearrangements of *Genesis*. Preliminary Southern analysis identified an abnormal Bam HI fragment in four of the eight tumors. The Bam HI sites flank the gene and the extra fragments indicate intragenic disruption of *Genesis*. Recurring cytogenetic abnormalities in malignancy have repeatedly provided a platform for gene identification as well as detection of the underlying biological mechanism leading to cellular deregulation and cancer. Further characterization of these abnormal fragments is underway.

<sup>1</sup>Sutton J, Costa R, Klug M, Field F, Xu D, Largaespada D, Fletcher C, Jenkins N, Copeland N, Klemsz M, Hromas R. *Genesis*, a winged helix transcriptional repressor with expression restricted to embryonic stem cells. *J Biol Chem* 271:23126-23133, 1996.

**A challenge to cytogenetic nomenclature and clinical diagnosis: Translocations, duplications and multiple insertions involving three chromosomes in a diagnostic leukemia.** *M.J. Sutcliffe<sup>1,2</sup>, D.P. Dumont<sup>1</sup>, L. Glaskova<sup>1</sup>, S. Gellatly<sup>1</sup>, R. Echevarria<sup>3</sup>, K. Washington<sup>1</sup>, P. Desai<sup>3</sup>.* 1) Cytogenetics, Dept of Pathology, All Children's Hosp, St Petersburg, FL; 2) Dept of Pediatrics, University of South Florida, Tampa, FL; 3) Depts of Hematology/Oncology and Pathology, St Anthony's Hospital, St Petersburg, FL.

A 68 year-old male patient was referred with a history of severe anemia and thrombocytopenia. Pathology revealed dysplastic features particularly of the erythroid series with less than 20% myeloblasts resulting in a diagnosis of myelodysplastic syndrome suggestive of refractory anemia with excess blasts (RAEB). Flow cytometry revealed bimodal expression of CD45 with a minor cell population expressing variable intensity myeloid associated antigens CD13, CD34 and HLA-DR. The majority of these cells were negative for CD33, however, dim CD33 may be present on a small population. Interpretation was a myelodysplastic or myeloproliferative disorder. Reasonable mitotic index and 400-450 GTG banding showed a pseudodiploid karyotype initially suggesting der(19)t(1;19), monosomy 12 and 21 plus two marker chromosomes. Of these cells, 40% also showed a deletion within 6q. Discrepant banding and fluorescence in-situ hybridization (FISH) using whole chromosome paint (WCP1) eliminated chromosome 1 involvement in the translocation with chromosome 19. Extensive FISH analysis using WCP, telomere and locus specific probes for chromosomes 12, 19 and 21 revealed a) three highly complex derivative rearrangements that include two duplications, three translocation and four insertions, and b) partial trisomy 12, trisomy 19 and tetrasomy 21. In the absence of FISH, these structural changes could not have been deduced. Even in the knowledge of the rearrangements, attempting to describe the karyotype almost defies the nomenclature possibilities of ISCN 95. Although trisomy 19 and 21 are associated with MDS, deletion 6q is usually characteristic of lymphoid malignancy. Reconciling the diverse cytogenetic outcome supportive of mixed lineage neoplasia with the pathology and flow cytometry indicating a myeloid "pre-leukemic" condition has resulted in clinical dilemma.



**Atypical FISH Signal Patterns with Chronic Myelogenous Leukemia.** *F.F. Yen<sup>1</sup>, J.H. Hersh<sup>1</sup>, M.J. Barch<sup>1</sup>, R.H. Herzig<sup>2</sup>, G.P. Herzig<sup>2</sup>, A. Ameri<sup>3</sup>, S. Bertolone<sup>3</sup>.* 1) Weisskopf Center for the Evaluation of Children/Dept. Pediatrics, Univ Louisville, Louisville, KY; 2) Dept.of Medicine/James Graham Brown Cancer Center, Univ Louisville, Louisville KY; 3) Pediatric Hematology-Oncology, Univ Louisville, Louisville, KY.

The hallmark of chronic myelogenous leukemia (CML) is the formation of a BCR-ABL fusion gene resulting from a Philadelphia chromosome (Ph) due to a balanced translocation involving 9q34 and 22q11.2. In 5-10% of patients with CML, the Ph cannot be identified cytogenetically, despite the presence of a submicroscopic rearrangement. Atypical FISH signal patterns occur occasionally, and their presence may provide clues to the clinical heterogeneity observed in affected individuals. We describe 2 patients with CML, in which only a single fusion signal on the derivative 22 chromosome was identified, using a LSI BCR/ABL es dual color dual fusion probe (Vysis cat.32-191032). In one, the cytogenetic study revealed a Ph, while in the other, the result was normal. In the patient with a Ph, we suspect that a deletion adjacent to the translocation breakpoint on the derivative 9 chromosome was present resulting in loss of one or more genes that may play a role in disease progression. In the cytogenetically normal patient, the FISH result reflected the presence of an interstitial insertion of the c-ABL oncogene at 22q11.2. Although reports of this finding suggest no effect on the clinical course, findings in both of these patients may prove to be important in further defining the heterogeneity and prognosis in CML.

**A highly unusual case of Acute Myeloid Leukemia (AML) M5a: Atypical clinical and cytogenetic findings.** *H.C. Rossbach<sup>1</sup>, L. Glaskova<sup>1</sup>, D.P. Dumont<sup>1</sup>, W. Chamizo<sup>1</sup>, J. Barbosa<sup>1</sup>, M.J. Sutcliffe<sup>1,2</sup>*. 1) Hematology/Oncology, All Children's Hospital, St Petersburg, FL; 2) Dept of Pediatrics, University of South Florida, Tampa, FL.

A 3 yr-old patient presented with a two week history of malaise, fatigue, intermittent low grade fever, bruising and leg pain. He developed massive bilateral proptosis with chloromas of the frontal cranial bones (reminiscent of metastatic neuroblastoma) and gingival hyperplasia. The clinical and pathological diagnosis of Acute Myeloid Leukemia subtype 5a suggested the non-random chromosome abnormality associated with AML M5a, i.e. t(9;11)(p21-22;q23). Translocation (9;11), however, was not observed. Cytogenetic analysis revealed a pseudodiploid karyotype involving two inversions within one copy of chromosome 11. The clone, comprising the majority of cells analyzed, also showed duplication 1q21q32. The remaining 7/20 cells showed a normal karyotype. Fluorescence In-Situ Hybridization (FISH) analyses using probe CCND1 for the 11q13 locus and the MLL probe at 11q23 clearly demonstrated the double rearrangement confirming the GTG banding result of pericentric inv(11)(p13q13) and paracentric inv(11)(q13q25). To our knowledge, the phenomenon of two inversions within one chromosome has not been previously described. The 11q deletion or translocation typically associated with M5 clusters around two breakpoint regions, 11q23-24 and 11q13-14. Two of three breakpoints in this case, however, are atypical. The 11q25 band is not specifically described in leukemia while the 11p13 band is usually associated with Acute Lymphoblastic Leukemia (ALL). In summary, although this patient has chromosome 11 rearrangements, the specific breakpoints and the double inversion appear to be a unique finding. At six months post sex mis-matched bone marrow transplant, only 25% of cells are derived from the donor. The current karyotype shows highly complex sub-clonal evolution. The significance of these karyotypic findings are reviewed in the context of the complicated clinical course.

**A Case of Undifferentiated Sarcoma with t(12;21)(q13;q11.2) and a history of Meningioma.** *K.H. Ramesh<sup>1</sup>, M. Edelman<sup>1</sup>, D. Wei<sup>1</sup>, R. Thomas<sup>1</sup>, E. Radel<sup>2</sup>, L.A. Cannizzaro<sup>1</sup>.* 1) Dept Pathology, Montefiore Medical Ctr/AECOM, Bronx, NY; 2) Department of Pediatric Oncology, Montefiore Medical Ctr., Bronx, NY.

The patient is an 18-year old female who had a several month history of right hip pain. MRI scan revealed a large right hemipelvic soft tissue mass centered within the pelvic and paraspinal muscles with extension through the right neural foramina of L4-L5 and L5-S1. Chest X-ray revealed bilateral pulmonary nodules. At age 9 years the patient had undergone resection of a spinal meningioma through an L3-L5 laminotomy. Open biopsy of the present pelvic mass revealed a "small round blue cell tumor". The tumor was immunoreactive for vimentin, neuron specific enolase, S-100 and BCL-2, but non-reactive with CD-99 (O-13), chromogranin, synaptophysin, GFAP, desmin, muscle specific actin and leukocyte common antigen. Although a diagnosis of PNET was considered, given the negative O-13 staining, the tumor was diagnosed as an undifferentiated sarcoma with neuroectodermal features. Cytogenetic information was not available on the meningioma. Chromosomal analysis of the undifferentiated sarcoma showed a 45,XX,del(9)(p13),t(12;21)(q13;q11.2),-22 karyotype. Normal cells were not seen. FISH analysis with whole chromosome paints 9,12,21 and 22 confirmed the cytogenetic diagnosis. Monosomy 22 is a characteristic cytogenetic change in meningioma and occurs less frequently in PNETs'. The deletion of 9p13 and translocation involving chromosomes 12 and 21 could be secondary changes indicating the evolving nature of the tumor. The immunoglobulin kappa J region recombination signal-binding protein-1 is on 9p13. The ERB-3 and human papillomavirus type 18 integration site-2 gene is on 12q13. The transient abnormal myelopoiesis gene is on 21q11.2. Is the current tumor related to the previous meningioma? Could the current tumor have resulted from dedifferentiation of residual tumor cells of the previous meningioma? Based on the occurrence of monosomy 22, it is reasonable to believe that the two lesions may in fact be related. After chemotherapy CT scan still shows the presence of lung nodules although there is significant reduction in size of the primary tumor. The prognosis for this patient is guarded.

**Molecular cytogenetic analysis of patients with acute lymphoblastic leukemia (ALL) utilizing multiple DNA probes for fluorescence in situ hybridization (FISH).** *L. Zhang, F. Bates, J.B. Parkhurst, J.J. Mulvihill, S. Li.*  
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The TEL/AML1 fusion results from a cryptic translocation between chromosomes 12 and 21 [t(12;21)(p13;q22)]. TEL/AML1-positive patients have exclusively B-cell lineage acute lymphoblastic leukemia (ALL) and a favorable prognosis compared with TEL/AML1-negative patients. However, the BCR/ABL fusion gene and MLL gene rearrangements are associated with an extremely poor outcome. These chromosomal rearrangements are too subtle to detect by conventional cytogenetic analysis, but can be readily detected using FISH techniques with commercially available DNA probes. We examined 33 patients with ALL for possible TEL/AML1, BCR/ABL fusion, and MLL rearrangements, 28 patients were pediatric patients (6 months to 19 years old) and 5 were adults (23 to 74 years old). We found that 9 out of the 28 pediatric patients (32%) were positive for TEL/AML1 fusion; all five adults were negative. All 9 patients with positive TEL/AML1 fusion had ALL pre-B cell lineage L1 according to standard immunotyping; eight were newly diagnosed leukemia, and one was in a second relapse 10 years after the first ALL. We also found that 4 pediatric patients, who were negative for the TEL/AML1 fusion, had an MLL gene deletion. All the patients lacked the BCR/ABL fusion. In agreement with previous reports, FISH testing using DNA probes specific for TEL/AML1 translocation should be a routine procedure for all patients with newly diagnosed ALL. The significance of MLL deletion found in 4 patients requires longer follow-up time to draw a definite conclusion.

**Application of molecular cytogenetic analysis (FISH) to identify a new translocation, t(11;17)(p13;q21.3), in a patient with acute myeloid leukemia.** *S. Shekhter-Levin*<sup>1,2,3,4</sup>, *S.S. Kaplan*<sup>3</sup>, *K. Cumbie*<sup>1</sup>, *M.E. Sherer*<sup>1,2</sup>. 1) Pittsburgh Cytogenetics Lab, University of Pittsburgh Center for Human Genetics at UPMC Magee Womens Hosp, Pittsburgh, PA; 2) Dept. of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; 3) Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, PA; 4) Univ. of Pittsburgh Cancer Institute, Pittsburgh, PA.

The patient, a 54-year old female, was diagnosed with acute myeloid leukemia (AML) FAB M5b a year ago and underwent four cycles of consolidation therapy six months ago. She now presents with blasts in her peripheral blood. Evaluation of her bone marrow revealed a hypercellular bone marrow consistent with AML, but the phenotype expressed no monocytoid characteristics noted at first presentation. Cytogenetic analysis of G-banded bone marrow cells, which had a normal karyotype previously, showed an abnormal mosaic chromosome pattern: 46,XX,del(17)(q23)[10]/46,XX[10]. FISH with the Vysis dual color *RARA* probe (17q21) and the Oncor dual color *MPO* probe (17q21.3-17q23) performed on metaphase cells showed intact *RARA* gene on the long arm of an abnormal chromosome 17, whereas the whole *MPO* gene was translocated from the abnormal chromosome 17 to the short arm of a C-group chromosome resembling an 11. Chromosome 11 and 17 paints confirmed the presence of a translocation between 11p and 17q, which was not identified by classical cytogenetic analysis. Consequently, the abnormal clone was designated as: 46,XX,t(11;17)(p13;q21.3).ish t(11;17)(p13;q21.3) (wcp11+,wcp17+,MPO+;wcp11+,wcp17+,RARA+,MPO-). The t(11;17)(p13;q21.3) translocation has not been reported previously, although translocations between 11q and 17q are well known in AML (M3 and M5 FAB types). Acquisition of this abnormal clone, which was not seen in this patient at the time of diagnosis, and the change in leukemia phenotype with loss of the monocytoid features suggest a possible association between these two events. It could be hypothesized that the transfer of the *MPO* gene from its normal position on 17q to 11p might lead to its dysregulation with consequent overexpression of the myeloperoxidase.

**Jumping translocations in childhood B-Cell malignancy.** *L.M. Pasztor<sup>1,2</sup>, L. Weems<sup>2</sup>, R. Callahan<sup>2</sup>, E. Kroman<sup>2</sup>, M. Recht<sup>3</sup>.* 1) Palo Verde Laboratory, A Division of Sonora Quest Laboratories, Tempe, AZ; 2) Clin Path Assoc., Tempe, AZ; 3) Section of Hematology/Oncology, Phoenix Children's Hospital, Phoenix, AZ.

Jumping translocations (JTs) and segmental jumping translocations (SJTs) are rare cytogenetic phenomena characterized by chromosomal amplification in which specific segments are translocated on to the ends of various chromosomes. JTs have been identified mostly in neoplastic conditions, although the first case reported in 1979 was constitutional. We report a case of Burkitt's lymphoma with peripheral involvement in a 2yr10month female. G-banding and chromosome painting characterized a 46,X,der(X)t(X;1)(q28;q21),t(8;14)(q24;q32) chromosome complement in each of the 20 bone marrow metaphases examined. In addition, there were several clones or individual cells with the donor 1q21->qter translocated to the telomeres of chromosomes 3,6,8,19 or 21. In effect, these JTs resulted in "partial tetrasomy" of the 1q segment. Interestingly, the leukemic cells were characterized as pre-B ALL(L1) by morphology and immunophenotyping.

Amplification of 1q by JTs has been reported in approximately 20 cases of acute myeloid and lymphoid leukemia and suggests that these rearrangements are secondary rather than primary cytogenetic events.

Our case supports the notion that a novel gene, perhaps JTB, at 1q21 fuses with telomeric sequences of receptor chromosomes to produce an aberrant transcript. The altered protein generated changes a critical biological feature leading to increased malignant potential of the tumor.

**Carney Complex: Cytogenetic Analysis of Tumors.** *L.D. Matyakhina<sup>1</sup>, S.D. Pack<sup>2</sup>, E. Pak<sup>2</sup>, J. Jaikumar<sup>2</sup>, P. Mannan<sup>2</sup>, L.S. Kirschner<sup>1</sup>, C.A. Stratakis<sup>1</sup>.* 1) DEB, NICHD, Bethesda, MD; 2) SNB, NINDS, Bethesda, MD.

Carney complex (CNC) is an autosomal dominant syndrome characterized by familial multiple neoplasia. The disease is heterogeneous and has been mapped to chromosomes 2p16 (CNC2) and 17q24 (CNC1). Mutations in tumor suppressor gene PRKAR1A (CNC1) were recently found in half of the CNC families, while the CNC2 gene remains unidentified. To elucidate the functional role of the CNC2 gene on 2p16, 36 tumors from CNC were studied by FISH using BAC probes from the 2p16 critical CNC region and BAC321G8 containing PRKAR1A gene. The most consistent finding from this analysis was amplification of the 2p16 region. The region of most consistent amplification is defined by BAC 400P14 (D2S2251-D2S2292), which showed the increased copy number in 20 of 26 touch preparations. A tandem duplication of dual color BAC in the interphase nuclei suggested the presence of homogeneously staining regions (HSRs). The degree of amplification varied from cell to cell within a single tumor suggesting an acquired instability process rather than monoclonal tumor cell expansion with an established amplicon. In 12 tumors this instability resulted in allelic deletion of 2p16 region. The changes of 2p16 region were observed in tumors from both groups of CNC patients with and without PRKAR1A mutations. However, in the latter case the loss of one PRKAR1A allele was revealed in 7 of 9 tumors (78%). The FISH analysis of 18 tumors from CNC patients with PRKAR1A mutations using BAC321G8 containing PRKAR1A gene showed the deletion of the 17q24 region in only 6 tumors (33%), suggesting that deletion of the normal allele on 17q24 is not necessary for tumorigenesis. Control experiments using FISH with the abovementioned BACs to normal adrenal cortex and BACs from other chromosomes to tumor cells revealed two signals of equal intensity in all cells examined. Taken together, our results suggest the presence of an oncogene on 2p16 that may be responsible for CNC in a subset of families. We speculate that activation of the CNC2 gene is triggered by inactivation or deletion of the CNC1 tumor suppressor gene or by amplification, which results in gain of function of the 2p16 gene independently of PRKAR1A.

**Evidence for deletion on chromosomes 6 and 12 in adenoid cystic carcinoma of the salivary gland.** *S. Rutherford*<sup>1</sup>, *R.M. Leu*<sup>1</sup>, *R. Saadut*<sup>1</sup>, *C.R. Marshall*<sup>1</sup>, *C.A. Rumpel*<sup>1</sup>, *W. El-Rifai*<sup>2</sup>, *S. Knuutila*<sup>3</sup>, *H.F. Frierson*<sup>1</sup>, *C.A. Moskaluk*<sup>1</sup>. 1) Dept of Pathology, University of Virginia, Charlottesville, VA; 2) Dept of Medicine, University of Virginia, Charlottesville, VA; 3) Dept of Medical Genetics, University of Helsinki, Helsinki, Finland.

Adenoid cystic carcinoma (ACC) is among the most common of malignant neoplasms that arise in salivary glands. The genetic alterations involved in the initiation and progression of ACC are not well characterized. Recently, we conducted comparative genomic hybridization (CGH) to characterize regions of genetic deletions in 24 cases of ACC. The results of this study showed loss at chromosome 6q (35% of ACC with DNA copy number changes) and at chromosome 12q (40%). To further investigate chromosomes 6 and 12, we assayed for loss of heterozygosity (LOH) using polymorphic microsatellite markers in paired tumor and normal samples of 58 cases. LOH results show hotspots of deletion occurring at the 6q24.2-q25.1 and 12q12-q13 regions. Results from LOH analysis also extended the CGH findings to show a small region of deletion occurring on the p arm of chromosome 12 indicating that one or more tumor suppressor genes may reside on this chromosome. Chromosome 12 deletion is infrequent in human neoplasms in general, and the underlying molecular defect(s) may be relatively specific for the tumorigenesis of ACC. Deletion of chromosome 6q has been described in ACC of the salivary gland, consistent with targeted inactivation of a tumor suppressor locus. Candidate tumor suppressor genes located within the chromosome 6 region of deletion include the pleomorphic adenoma of the salivary gland gene like 1 (PLAGL1/LOT1/ZAC), tumor suppressor-activated pathway 21 (TSAP21) and the large activating tumor suppressor gene (LATS1). Our results provide additional evidence for the existence of a deleted region on 6q, which may harbor one or more tumor suppressor genes important in the development of salivary gland carcinoma.



**Reciprocal Translocation 46,XY, t(1;4)(q11;q11), in a Gigantiform Cementoma. A case report.** *J.M. Aparicio<sup>1</sup>, M.P. Barrientos<sup>1</sup>, M.L. Hurtado<sup>1</sup>, B. Molina<sup>2</sup>, S. Frias<sup>2</sup>, C.F. Salinas<sup>3</sup>.* 1) Dept Genetics, Hosp para el Nino Poblano, Puebla, Mexico; 2) Dept of Cytogenetic, Inst Nacional de Pediatria, Mexico city; 3) Craneofacial Genetics, Med Univer of South Carolina, USA.

**INTRODUCTION.** *A gigantiform cementoma (GC) is a fibro-osseous tumoral lesion of Jaws, a rare entity with a dominant autosomic inheritance. Despite the reported association of tumoral lessions with autosomic chromosomal translocations, this chromosomal aberration have not been found in GC.* **CASE REPORT.** *An adolescent male with a fibro-osseous lesion of the jaws, resembling a GC clinically, radiologically and histopatologically; in addition with a flat fascies and minor phenotypic dismorfies is presented. The karyotype showed the existence of a de novo reciprocal translocation between the long arms of chromosomes 1 and 4.* **CONCLUSION** *This is the first report of a GC related to the precense of this specific genetic abnormality, consisting in a reciprocal nHCT due to a cytogenetic mutation. it was though that part of the centromere and chromosome 4 short arm was included, at the beggining of this study. However, by using fluorescence methods (FISH), this probability was eliminated. Clinical records, radiological images and histopatholgical finfings are commented, emphasizing the relevance of the genetic study and counseling in this and other entities with tumor growth, that have been related to chromosome translocations in pairs 1 and 4.* **KEEWORDS:** *Chromosome 1 and 4, gigantiform cementoma, chromosomal translocation, karyotype..*

**ENDOMETRIAL CYTOGENETIC EVALUATION IN HEALTHY WOMEN THAT INGESTING ORAL CONTRACEPTIVE.** *A.E. Rojas-Atencio<sup>1</sup>, Z. Villalobos-Vega<sup>2</sup>, K. Urdaneta<sup>1</sup>, G. Garcia<sup>1</sup>, L. González<sup>2</sup>, M. Soto-Alvarez<sup>1</sup>, F. Alvarez Nava<sup>1</sup>.* 1) Unidad de Genetica, Univ del Zulia, Maracaibo , Zulia, Venezuela; 2) Hospital Clinico de Maracaibo.

The oral contraceptives constitute the birth-control method more used by the women that want to maintain their capacity to conceive in a future. The objective of this study was to evaluate the chromosomal findings in endometrio of healthy women, assisted to the family planning, during the period of august to December of 2000. Twenty-five women were chosen at random among 18 to 40 years that ingested oral contraceptive for a time by 10 years with or without risk factor to neoplasia (obesity, smokier, gonadal dysfunction, etc) Only 12 endometrial samples were available for cytogenetic study. We were used technical of short culture and GTG banding. Of the 12 analyzed samples, 9 (75%) presented normal chromosomal features. Chromosomal aberrations was observed in 3 women (25%), being evidenced risk factors associated with the type and time of consumption of the contraceptive, obesity, smokier, polycystic ovaries, chronic anovulation, and endometrial hyperplasic. Were observed, monosomy 10, 12 and 14, endoreduplications, fragile sites specially in 11q23, tetraploidy, marker chromosome and translocation (8q;15q). These findings suggest that the presence of chromosomal anomalies associated with the consumption of oral contraceptive could be used as an indicative of beginning of endometrial adenocarcinoma. These cytogenetic anomalies have been reported in-patient with this pathology. We conclude that all patient with consupcion of oral contraceptive uninterruptedly for one year should be practiced besides their routine studies, an chromosomal analysis to detected anomalies that could be compatible precociously with the beginning of the cancer.

**Molecular heterogeneity of MLL/AF17 fusion transcripts in acute leukemias.** *S. Strehl<sup>1</sup>, M. König<sup>1</sup>, O.A. Haas<sup>1,2</sup>.*  
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The t(11;17)(q23;q21) accounts for 1-1.5% of all MLL rearrangements and results in a chimeric transcript that fuses the 5' end of the MLL gene to the 3' end of the AF17 gene. However, at the molecular level only one case of an MLL/AF17 fusion has been described. We have identified 10 patients with acute leukemia carrying a t(11;17)(q23;q21-25) by cytogenetics and/or chromosome painting, and in 9/10 rearrangement of the MLL gene was detected by FISH. Further FISH analysis using gene-specific probes suggested the presence of an MLL/AF17 fusion in 5 patients. Performing RT-PCR with published primers failed to identify the fusion transcript in 4/5 patients. Therefore, further RT-PCRs using various primer combinations were performed, and in 4 patients 4 new fusion transcript variants were detected. Two patients displayed fusion of MLL exon 8 to AF17 nt 1942 of the translated region. In one of them, an additional alternatively spliced MLL/AF17 transcript was co-expressed. In this transcript, MLL exon 8 was fused to a hitherto undefined AF17 exon. Alternative splicing of the AF17 gene in peripheral blood was confirmed by exon-specific RT-PCR. In the third patient MLL exon 9 was fused to another alternatively spliced AF17. In this case, the MLL gene fused to nt 552 of AF17, and exclusion of a 615-bp exon was detected. In the fourth patient an unusual combination of exon sequences in the chimeric transcript was observed. Both the MLL and the AF17 genes were fused within exons, namely MLL exon 10 and AF17 nt 1745. In this instance it remains to be determined whether this fusion variant is due to the activation of cryptic splice sites or whether the genomic breakpoints occur within exons. In all instances the open reading frames were maintained without frameshift, thus resulting in true fusion proteins. All predicted MLL/AF17 chimeric proteins included the most notable feature of the AF17 protein, a leucine-zipper dimerization motif. The high heterogeneity of MLL/AF17 fusion transcripts emphasizes the importance of appropriate primer selection for RT-PCR analysis in diagnostic settings.

**Multicolor FISH analysis in ten patients with acute lymphoblastic leukemia.** *G. Calabrese*<sup>1,2</sup>, *T. Taraborelli*<sup>1,2</sup>, *D. Fantasia*<sup>1,2</sup>, *E. Morizio*<sup>1,2</sup>, *P. Guanciali Franchi*<sup>1,2</sup>, *V. Gatta*<sup>1</sup>, *A. Spadano*<sup>3</sup>, *L. Stuppia*<sup>1,4</sup>, *G. Palka*<sup>1,2</sup>. 1) Dip S. Biomed/Genetica Univ DG Annunzio, Chieti, Italy; 2) Serv. Genetica U., Osp. Civ. Pescara, Italy; 3) Dip. Ematologia, Osp. Civ. Pescara, Italy; 4) Ist. Citomorfologia N&P, CNR, Chieti, Italy.

Spectral karyotyping (SKY), and multicolor FISH banding analyses allow precise identification of subtle translocations, marker chromosomes, and complex chromosomal aberrations. At present few data have been reported in acute lymphoblastic leukemia (ALL) patients using these approach. We investigated 10 ALL patients using both multicolour FISH approaches. In four patients SKY analysis confirmed normal karyotype shown by conventional G-banding. In one patient showing 15% of diploid cells with del(17)(p12) and p53 gene loss by FISH, SKY was unable to detect any anomaly likely since intrachromosomal rearrangement are not suitable for spectral investigation. In the other five patients with two or more chromosomal abnormalities SKY analysis showed in two cases the same anomalies identified by standard cytogenetics while karyotypes were precisely refined in three cases. In a T-ALL patient, a t(12;20)(p13;q12) was redefined by SKY as t(11;20)(p13;q12) with rearrangement of genes involved in hematopoietic pathway. In another patient, with a Common subtype, SKY reclassified an add(7) as der(7)t(7;8)(q3;?), a rare rearrangement in ALL. Using multicolor banding probe for chromosome 7 a deletion on 7q34-35 was also detected unraveling a cryptic more complex abnormality. In the remaining patient, with a T cell ALL, SKY analysis identified a marker chromosome as dic(1;9)(p36;p11-13) with loss of tumor related genes on 1p36 and 9p. Moreover SKY analysis also disclosed a cryptic t(10;11)(p13;q21) which harboured a specific CALM/AF10 fusion gene responsible for poor clinical outcome. In conclusion, in three cases with multiple rearrangements SKY and multicolor banding using the same digital multifuorescence apparatus resulted in a rapid cost-effective approach which allowed us to refine abnormal karyotypes and to detect cryptic rearrangements related to adverse prognosis.

**Deletions of 13q14 are significant in the diagnosis of multiple myeloma.** *C.M. Higgins, H. Blair, B. Dave, W.G. Sanger.* Human Genetics Laboratory, Univ Nebraska Medical Ctr, Omaha, NE.

Multiple myeloma (MM) is a lymphoproliferative disorder characterized by the malignant proliferation of monoclonal plasma cells. Because of the low proliferative rate of malignant plasma cells, cytogenetic abnormalities are often difficult to ascertain. Recently, FISH techniques have helped to establish a more cogent relationship between a deletion of 13q14 & the poor clinical course of this disease. Our laboratory routinely screens newly diagnosed MM patients with a DNA probe for 13q14 as an adjunct to conventional cytogenetics. This study summarizes our experience in detecting cytogenetic abnormalities & deletions of 13q14 by FISH analysis. One hundred-eight newly diagnosed MM cases were studied in our laboratory utilizing conventional cytogenetics & interphase FISH with a DNA probe specific for 13q14. Eighty-two of these cases were found to be normal by cytogenetics & by FISH. Twenty-six cases (24%) were abnormal by cytogenetics &/or by interphase FISH analysis of 13q14. Sixteen cases (15%) showed a deletion of 13q14 by FISH; 6/16 cases contained normal karyotypes by G-band analysis; 10/16 cases were cytogenetically abnormal & 8 of these cases had either a visible deletion or monosomy 13. Twenty cases (19%) with abnormal karyotypes included 10 cases without 13q14 involvement & 10 cases with deletion 13q14 (by FISH). Besides chromosome 13, which was most frequently altered, chromosomes 5, 7 & 9 were most predominantly observed as numeric abnormalities (9/20; 45%, each). Structural abnormalities frequently involved chromosomes 1 (6/20; 30%) & 11 (4/20; 20%). All 4 cases with structural abnormalities of 11 had involvement of the 11p15 region. A recurrent translocation, t(1;11)(p13;p15), was observed in 2 cases. FISH studies provided a valuable tool to complement conventional cytogenetics in detecting higher number of abnormalities in MM. A combination of interphase FISH screening for 13q14 deletions & routine cytogenetic analysis in MM patients at diagnosis may prove to be beneficial in determining the prognosis & in designing an effective therapeutic strategy for newly diagnosed MM patients.

**Quantitative PCR and IHC staining for HER-2/neu. Retrospective comparative case study of breast cancer specimens selected for chromosome 17 aneusomy by FISH.** *I. Simonic<sup>1,2</sup>, B. Manasse<sup>1</sup>, S. Smocilac<sup>1</sup>, G. Stipinovich<sup>1</sup>, P. Lombard<sup>1</sup>, T. Slavik<sup>1</sup>.* 1) Ampath Group, Pomona Rd. 29, Johannesburg, South Africa; 2) Medical Research Council, MRC, Southpansberg Rd. 1, Pretoria, South Africa.

In total 57 cases pre-selected due to ambiguous HER-2/neu IHC result were followed up by FISH analysis in our laboratory to date. 13/57 cases were identified positive for chromosome 17 aneusomy and 3/57 cases were clearly positive for penta- and hexasomy of chromosome 17, without the actual HER-2 amplification. The IHC staining was repeated on the later three specimens and followed by quantitative PCR for HER-2/neu amplification. Comparative results are presented and their clinical significance discussed with respect to the management of the patients. Moreover, two additional cases were identified in the course of the clinical HER-2/neu FISH analysis with augmented CEP17 copy number with accompanying HER-2/neu amplification.

**Detection of a variant translocation of p190 *BCR-ABL* by FISH and Multiplex RT-PCR with multicolor fragment analysis, including detection of minimal residual disease.** *J.F. Pulliam*<sup>1</sup>, *D.A. Jezek*<sup>1</sup>, *P.P. White*<sup>1</sup>, *J.A. Moscow*<sup>2</sup>, *C.D. Jennings*<sup>1</sup>, *A.L. Pettigrew*<sup>1</sup>. 1) Dept Pathology, Univ Kentucky, Lexington, KY; 2) Dept Pediatrics, Univ Kentucky, Lexington, KY.

A patient presented with acute lymphoblastic leukemia (WBC of 48,000 with 95% blasts and bone marrow replaced by FAB-L2 blasts). Karyotyping at diagnosis showed a t(9:22)(q34.1;q11.2) with multiple secondary changes. A multiplex RT-PCR method with reverse-dot blot confirmation to allow the detection of all known variants of 5 common acute lymphocytic translocations (p190 and p210 *BCR-ABL*, *ETV6-AML1*, *AF4-MLL*, *PBX1-E2A*) originally described by Scurto P, et al (*Leukemia* 12:1994 (1998)) was modified with multicolor fluorescent primers and multicolor fragment analysis to allow detection on the ABI 310 Genetic Analyzer. Application of multiplex RT-PCR to this case identified a p190 *BCR-ABL* translocation.

At 10 months post remission induction, bone marrow fluorescent *in situ* hybridization (Vysis LSI *BCR-ABL* ES Dual Color Probe) and flow cytometry showed minimal residual disease (2.6% cells with a variant *BCR-ABL* fusion and the diagnostic leukemic immunophenotype admixed with 13% normal B-lineage precursors (hematogones)). At 12 months and following delayed intensification, no evidence of the clone was identified.

This case demonstrates the successful adaptation of the multiplex RT-PCR technique with multicolor fragment analysis to the detection of ALL translocations, and the utility of minimal residual disease detection with complementary modalities.

Scurto P, Hsu Rocha M, Kane JR, Williams WK, Haney DM, Conn WP, Shurtleff SA, Downing JR. A multiplex RT-PCR assay for the detection of chimeric transcripts encoded by the risk-stratifying translocations of pediatric acute lymphoblastic leukemia. *Leukemia*, 1998 Dec; 12(12):1994-2005.

**Evidence For Genetic Anticipation in Familial Prostate Cancer.** *C.H. Bock<sup>1</sup>, P.A. Peyser<sup>1</sup>, K.L. Tedesco<sup>2</sup>, C.E. Mohai<sup>2</sup>, K.A. Cooney<sup>2,3</sup>.* 1) Department of Epidemiology, University of Michigan School of Public Health; 2) Department of Internal Medicine, University of Michigan Medical School; 3) Department of Urology, University of Michigan Medical School, Ann Arbor, MI.

Decreased age of onset in successive generations has been observed for a number of diseases with a genetic basis. Participants in the University of Michigan Prostate Cancer Genetics Project were studied to determine whether differences in age of diagnosis within vertical generations, a hallmark of genetic anticipation, exists in familial prostate cancer. Families with at least one affected father-son pair were included in the study, resulting in 309 pairs from 191 families (508 total individuals, 11% black). Average age at diagnosis among sons and fathers was 58 (sd=9.1, range = 35,81), and 72 years (sd=8.9, range = 45,93) respectively. The average difference in age at diagnosis (14 years) was highly statistically significant by a paired t-test ( $t=20.58$ ,  $p<.0001$ ). To reduce detection bias in the sample, we restricted the analysis to the 120 pairs in which the son was born prior to 1936. The statistically significant difference in age at diagnosis persisted ( $t=6.54$ ,  $p<.001$ ). Analyses were repeated in the 136 pairs that did not include the proband to reduce ascertainment bias in the sample and a highly statistically significant difference in age at diagnosis was still observed ( $t=9.33$ ,  $p<.0001$ ). Significant results were also detected in both black and white subgroups when the data was stratified by race. Study findings were supported by life table analyses, in which age at diagnosis in sons was found to be significantly younger than age at diagnosis in fathers when the Gehan's Wilcoxon generalized test statistic was computed for the entire sample. Differences in ages of diagnosis remained when the analysis was restricted to individuals born prior to 1936, when probands were excluded, and after stratification by race. Taken together, there is evidence that sons of men with prostate cancer have an earlier age of diagnosis than their fathers, which suggests anticipation. This finding, if confirmed, will have important implications for risk assessment in familial prostate cancer.



**Variants in the genes that encode the BRCA1-associated genome surveillance complex (BASC) in BRCA1 mutation carriers.** *K.L. Nathanson<sup>1,3</sup>, R. Letrero<sup>1</sup>, P.A. Kanetsky<sup>2</sup>, R. Omaruddin<sup>1</sup>, C. Issacs<sup>4</sup>, C. Lerman<sup>3</sup>, T.R. Rebbeck<sup>2,3</sup>, B.L. Weber<sup>1,3</sup>.* 1) Dept Medicine, Abramson Family Cancer Research Institute; 2) Dept Epidemiology & Biostatistics; 3) Cancer Center, Univ Pennsylvania Sch of Med, Philadelphia PA; 4) Lombardi Cancer Center, Georgetown University, Washington DC.

Mutations in BRCA1 confer a greatly increased risk of breast cancer. However, the breast cancer risk due to mutations in BRCA1 varies from 40-85% depending on the population ascertained. Therefore it has been speculated that the mutation alone does not explain the observed phenotype and that other factors, including variants in genes other than BRCA1 and BRCA2, influence the development of breast cancer in BRCA1 mutation carriers. As BRCA1 and BRCA2 function in DNA damage response pathways, variants in other DNA damage response genes that are associated in complexes with BRCA1 and BRCA2 are particularly good candidate genes to modify penetrance in BRCA1 mutation carriers. We have selected variants in genes that are part of the BASC complex (MLH1, MSH2, MSH6 and ATM) as candidate genes in which single nucleotide polymorphisms may modify penetrance in BRCA1 mutation carriers. Using 221 female BRCA1 mutation carriers we studied the following variants: ATM - 5'UTR 10805 A/G, D1853N, MLH1 - 5'UTR -93 G/A, MSH2 - IVS9 -9 T/C, IVS12 -6 T/C, and MSH6 - G36E. Female BRCA1 mutation carriers were stratified by whether or not they had a breast cancer diagnosis. Of the 6 variants, only the presence of the C at position -9 in IVS9 of MSH2 may be associated with the diagnosis of breast cancer (OR=1.9, 95% CI 0.97-3.6). As BRCA1 and MSH2 function in different pathways of DNA damage repair, double strand break repair and mismatch repair respectively, it is possible that alterations in multiple pathways may be more important than multiple alterations in the same pathway.

**Suppression of tumor formation by expression of semaphorin 3F in HEY ovarian carcinoma but not in GLC45 small cell lung cancer cells.** *S.L. Naylor*<sup>1</sup>, *A.R. Davalos*<sup>2</sup>, *C.H. Hensel*<sup>3</sup>, *RH. Xiang*<sup>1</sup>. 1) Cellular & Structural Biol, Univ Texas Health Science Ctr, San Antonio, TX; 2) Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Myriad Genetics, Salt Lake City, UT.

Loss of heterozygosity on human chromosome 3p21.3 is a frequent occurrence in many tumor types. In a previous study, our laboratory demonstrated functionally that an 80 Kb P1 clone from chromosome 3 suppresses the tumorigenicity of the mouse fibrosarcoma cell line A9. Two cDNAs corresponding to genes encoded on this P1 clone, semaphorin 3F and N23, were tested for their effects on in vitro and in vivo growth characteristics following transfection into mouse A9 cells. Transfection of semaphorin 3F cDNA resulted in complete loss of tumorigenicity in nude mice whereas transfection of N23 had no effect. Moreover, semaphorin 3F also functioned to block apoptosis of transfected A9 cells treated with taxol or adriamycin. To test whether SEMA3F had an effect on human tumors, we transfected the small cell lung cancer line GLC45. Expression of semaphorin 3F in this cell line had neither in vitro nor in vivo effects. Human ovarian cancer also has a high rate of loss of heterozygosity. Upon transfecting the human ovarian adenocarcinoma cell line HEY, we found loss of the capacity for growth on soft agar. Moreover, expression of SEMA3F in HEY cells resulted in the suppression of tumor formation in nude mice. Our results indicate that the effects of semaphorin expression on in vitro and in vivo growth have a cellular specificity.

**Genetically induced pituitary tumors in POMC null mouse mutants.** *J. Costa*<sup>1</sup>, *G.Y. Li*<sup>2</sup>, *B.R. Robinson*<sup>2</sup>, *A.F. Parlow*<sup>2</sup>, *U. Hochgeschwender*<sup>2</sup>, *M.B. Brennan*<sup>3</sup>. 1) Human Medical Genetics Program, University of Colorado Health Sciences Center, Denver, CO; 2) Oklahoma Medical Research Foundation Oklahoma City, OK; 3) Eleanor Roosevelt Institute, Denver, CO.

POMC null mutant mice lack all POMC-derived peptides and recapitulate the phenotypes of obesity, pigmentation defects, and adrenal insufficiency as observed in human patients with POMC gene mutations. An additional phenotype observed in the mice is the occurrence of pituitary tumors in both homozygous and heterozygous mutants. By the age of 5-7 months > 90% of these mice begin to express symptoms of pituitary tumors, such as weight loss, and die by 7-9 months of age. Immunohistochemical examination revealed the tumors to be similar to non-functional macroadenomas in human patients: tumor tissues appear non-secreting after staining with TSH, PRL, GH, ACTH, -MSH, and LH, while the normal parts of the pituitaries produce the expected hormones. Sera of these mice were also evaluated for the presence of pituitary hormones with no significant elevation in hormones tested (GH, PRL, TSH). The involvement of POMC mutations as genetic factors in pituitary tumors was unexpected. However, as POMC null mutations are known to occur naturally in man and as clinically nonfunctioning pituitary adenomas comprise approximately 25-30% of human pituitary tumors, the etiology of pituitary tumors in POMC null mice may be relevant to human pituitary neoplasia. We are currently testing such human adenomas for the presence of POMC mutations.

**Frequent upregulation of Wnt signaling pathway in human pituitary adenoma.** *T. Aiba<sup>1</sup>, S. Semba<sup>1</sup>, C. Sun<sup>1</sup>, M. Imai<sup>1</sup>, T. Furukawa<sup>1</sup>, H. Ikeda<sup>2</sup>, A. Horii<sup>1</sup>.* 1) Department of Molecular Pathology, Tohoku University School of Medicine, Sendai, Miyagi, 980-8575 Japan; 2) Department of Neurosurgery, Tohoku University School of Medicine, Sendai, Miyagi, 980-8575 Japan.

Upregulation of the Wnt signaling pathway is playing an important role in cell proliferation. Several proteins such as APC, beta catenin (CTNNB1), GSK3beta, AXIN-1 and -2, and ICAT are known to be involved in regulation of the Wnt pathway. Recently, MYC and cyclin D were found to be the target downstream genes in the Wnt signaling pathway. As we previously observed over expression of MYC in human pituitary adenomas, it is of great interest in analyzing molecules involved in regulation of the Wnt signaling pathway. A total of 37 pituitary adenomas were analyzed in this study. Expression of CTNNB1 and cell proliferation marker Ki-67 were studied immunohistochemically. Twenty-one (57%) of 37 pituitary adenomas demonstrated abnormal nuclear accumulation of CTNNB1. Mutations in the CTNNB1 gene were found in only four tumors. We further searched for mutations in the other genes such as APC, GSK3beta, AXIN-1 and -2, and ICAT, but none of the mutations were observed. These results suggest that (1) upregulation of the Wnt signaling pathway is playing an important role, and (2) unknown molecule(s) other than CTNNB1 is playing an important role(s) in human pituitary tumorigenesis.

**A physical transcriptome map for chromosomal analysis of lung cancer microarray data.** *A.M. Levin, A.M. Levin, S.L.R. Kardia.* Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI.

One important application of the current draft sequence is the development of a transcriptome map of the genes in the human genome. Cytogenetic, genetic, and radiation hybrid maps have been developed, and now with the draft sequence, we have the means to generate a physical transcriptome map of human chromosomes. We used the cDNA sequences present on the Affymetrix HuGeneFL Array as a base to develop such a map and apply it to perform chromosome level gene expression analyses. Using the Basic Local Alignment Tool (BLAT) and the Golden Path draft sequence construction (developed by Kent and Haussler, <http://genome.ucsc.edu/>), we were able to place 91.2% (n=6504) of the 7129 cDNAs present on the oligoarray. The largest query sequence alignment was used to determine the starting and ending positions within chromosomes. 83.8% (n=5451) of the 6504 cDNAs aligned within single chromosomes. We employed the Unigene and LocusLink curated databases at the National Center for Biotechnology Information to align the remaining sequences. The position of 16.2% (n=1053) of the remaining cDNAs were resolved using information from these data sources. The chromosome information provided by the BLAT search and Unigene/LocusLink conflicted for 10.3% (n=671) of the 6504 cDNAs placed. The positions given by BLAT were used in these cases. The remaining 8.8% (n=625) of the original 7129 cDNAs had no annotated information with which to infer chromosomal location and therefore remained unpositioned. We are currently developing analytical tools to analyze chromosome level gene expression profiles to identify patterns that differentiate between stage I and stage III lung tumors.

**Tree-based methods for tumor classification with gene expression microarray.** *H.P. Zhang<sup>1</sup>, C.Y. Yu<sup>1</sup>, B.H. Singer<sup>2</sup>, M.M. Xiong<sup>3</sup>.* 1) Dept Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT; 2) Princeton University, Princeton, NJ; 3) University of Texas, Houston, TX.

Precise classification of tumors is both critically important for cancer diagnosis and treatment, and scientifically challenging. Recent studies have shown the improved precision of classification by using gene expression profiles. However, the success has been limited by analytic methodologies. Using a published data set for purposes of comparison, we introduce a methodology based on classification trees and demonstrate that it is significantly more accurate for discriminating among distinct colon cancer tissues than other statistical approaches used heretofore. In addition, competing classification trees are displayed. They suggest that different genes may co-regulate colon cancers.

**Comprehensive Gene Expression Profile of the Matrix Metalloproteinase (MMP) Gene Family in Normal Human Tissue and Major Cancers.** *G.L. Shen*<sup>1</sup>, *J. Cossman*<sup>1, 2</sup>. 1) Pharmacogenomics, Gene Logic, Inc, Gaithersburg, MD; 2) Georgetown University, Washington DC.

The MMPs are a large family of proteinases involved in the degradation of the extracellular matrix proteins. There has been a growing interest in the design and evaluation of MMP inhibitors as anticancer agents because a variety of malignant tumor types had been shown to overexpress MMPs. Gene expression profiles of 350 normal and tumor tissues were generated using the Human U95 Affymetrix GeneChip array platform for simultaneous analysis of 60,000 fragments, with a transcript coverage of 12,000 Full Length Genes and 48,000 Unigene ESTs. Fold-change analysis comparing normal and tumor samples from breast, ovarian, uterine, lung, colorectal, skin and pancreatic tissues were performed. Between 10-30 samples were used for each tissue set. Principal component analysis (PCA) was performed on samples from each tissue type to investigate global differences in expression profiles between tumor and normal samples. The normal and tumor-derived samples could be separated by PCA into 2 distinct populations based upon their respective gene expression patterns. The levels of expression of the various members of the MMP gene family varied significantly across the different tissue sets, ranging from undetectable expression in many of the normal tissue types to different but significant levels of increased expression in the different tumor types. For example, in comparison to normal tissue from the corresponding organ, MMP12 was upregulated, by 2-3 fold (p-value<0.01) in breast and colorectal cancer and by over 10 fold (p-value<0.01) in lung cancer. In silico, electronic-Northern analysis of more than 4000 human tissue samples of over 30 tissue types revealed that MMP2, MMP11 and MMP14 are expressed by >70% of human tissues, whereas MMP3, MMP10 and MMP13 are found to be expressed in <15% in the same tissues. The degree of expression of any MMP over a broad range of human tissue types provides a basis for designing specific MMP inhibitors and predicting their potential risk of toxic events at these anatomic sites.

**Loss of heterozygosity at the EXT and EXT-like loci in osteochondromas and secondary chondrosarcomas of hereditary multiples exostose patients.** *L. Legeai-Mallet, F. Noel, A. Cohen-Tanugi, C. Benoist-Lasselin, A. Munnich, J. Bonaventure.* Genetique INSERM U393, Hopital Necker, Paris, France.

Hereditary Multiple Exostoses (HME) is an autosomal dominant disorder characterized by ectopic growth of osteochondromas (exostoses). The malignant transformation of osteochondromas into chondrosarcomas occurs in 5% of cases. HME is genetically heterogeneous as three loci have been mapped to chromosome 8q24 (EXT1), 11p11-13 (EXT2) and 19p respectively (EXT3). Three additional loci designated EXTL1, EXTL2 and EXTL3 have been also identified and mapped to chromosome 1 (1p36 and 1p11-p12) and 8 respectively (8p12). We searched for loss of heterozygosity (LOH) at the EXT and EXT-like loci in eight hereditary osteochondromas and three secondary chondrosarcomas from 8 HME patients carrying EXT1 (5/8) and EXT2 (1/8) germline mutations. Microsatellite analysis at the EXT and EXT-like loci revealed that 5/8 osteochondromas and 3/3 chondrosarcomas displayed LOH at EXT and/or EXT-like loci. LOH was mostly found at the EXT1 locus (6/11, 54%) but also at the EXTL2 (2/11, 18%), EXT3, EXTL1 and EXTL3 loci (1/11, 9%). Multiple LOH at the EXT1, EXT3, EXTL1, and EXTL3 loci was found in a EXT1 patient harboring two osteochondromas and two chondrosarcomas. Neither LOH nor additional EXT mutation were detected in osteochondromas from two HME patients carrying EXT1 germline mutations. These results indicate that multiple LOH occur during the malignant transformation of osteochondromas and that LOH is not necessarily allelic of the germline EXT mutation. They also suggest that two hits at the EXT or EXT-like loci (one germline mutation in one EXT gene and one somatic event) are required for osteochondromas to undergo malignant degeneration.



**1p/19q deleted oligodendroglioma is a distinct histological tumour.** *C. Godfraind*<sup>1</sup>, *E. Rousseau*<sup>1,2</sup>, *C. Raftopoulos*<sup>3</sup>, *M. Ruchoux*<sup>4</sup>, *F. Scaravilli*<sup>5</sup>, *M. Vikkula*<sup>2</sup>. 1) Division of Neuropathology, Univ. catholique de Louvain, Brussels, BELGIUM; 2) Lab. of Human Molecular Genetics, Christian de Duve Institute & Univ. catholique de Louvain, Brussels, BELGIUM; 3) Division of Neurosurgery, Univ. catholique de Louvain, Brussels, BELGIUM; 4) Department of Neuropathology, Hopital R. Salingro, Lille, France; 5) Institute of Neurology, London, UK.

Oligodendroglioma is a tumour originating from oligodendrocytes, the myelin forming cells in the central nervous system. This glioma preferentially occurs in adults. It is mostly located in cerebral hemispheres with a predilection to the frontal lobe. This lesion accounts for 5-33% of all gliomas. The wide range reported for tumour occurrence reflects inter-observer discordance in histological diagnosis. Genetic analysis of oligodendroglioma has associated 1p/19q-deletions to chemosensitivity. No histological definition for this subgroup has been reported. In order to correlate 1p/19q-deleted oligodendroglioma to a histological phenotype, we underwent a retrospective study of 59 formaline fixed and paraffin embedded gliomas, mostly reported as oligodendroglioma in the Neurological Institute, London and in the Cliniques Universitaires St Luc, Brussels. Chromosomes 1, 9, 10, 17, 19 and 22 were studied by microsatellite analysis in order to define regions of loss of heterozygosity (LOH). A total of 22 1p/19q-deleted gliomas were identified. Twenty of them share common characteristic histological criteria. This allows to diagnose 1p/19q-deleted oligodendroglioma on histological criteria prior to any genetic analysis. Thus, a better identification of glioma subtypes and evaluation of their treatment can be done even in centres without genetic diagnostics. (vikkula@bchm.ucl.ac.be).

**Type 1 Neurofibromatosis 1: retrospective neuroradiologic evaluation of 79 cases.** *M.P. Fondelli<sup>1</sup>, S. Costabel<sup>2</sup>, S. Massa<sup>2</sup>, D. Sambarino<sup>2</sup>, M.L. Garre<sup>4</sup>, L. Andreussi<sup>3</sup>, C. Bellini<sup>2</sup>, E. Bonioli<sup>2</sup>.* 1) Neuroradiology; 2) Oncology; 3) Neurosurgery Departments - G. Gaslini Children Hospital, Genova; 4) Pediatrics Department, University of Genova, Italy.

A series of 79 patients affected by Type 1 Neurofibromatosis has been retrospectively evaluated from a neuroradiologic point of view. The patients have performed a neuroradiologic follow-up from 1988 to 2000. In all patients the diagnosis was performed on NIHCC criteria: in about 50% of them a molecular diagnosis with truncated protein test has been accomplished. All patients underwent a cranial MRI, according to the standard protocol for Type 1 Neurofibromatosis. The aim of this work is to focus on the neuroradiologic features of this disease, particularly the more unusual ones, stressing their clinical implications. The following findings have been observed: a *megalic corpus callosum*, due to the reduction of the glial apoptosis; this feature has also been observed in other inheritable hamartomatous syndromes, due to tumour suppressor genes spontaneous mutation, like the Bannayan Zonana Syndrome; *dural ectasies*, variously associated with osseous scalloping and more frequently located around the brainstem; *non evolutive optic nerve swelling*, at this regard a differential diagnosis must consider the simple optic nerve dural sheath ectasia, the perioptic arachnoidal gliomatosis and the true optic nerve glioma; *UBOs and histogenetic foci*, reflecting a wide spectrum of maturational and structural glial cells anomalies, where both the transient aspect and the variable proliferative potential must be considered; *true tumours* involving the optochiasmatic tract and others encephalic regions; *cystic astrocytoma-like non evolutive foci*; *associated cerebral malformations*, like Chiari 1, cortical dysplasias, megacisterna magna, arachnoidal cysts, etc. In a case on MRI a complicated mixture of type 1 neurofibromatosis and tuberous sclerosis features was observed. The neuroradiologic MRI evaluation confirms a great variability of cerebral manifestations in patients affected by Type 1 Neurofibromatosis, according to the proteiform clinical picture of the disease.

**Molecular characterization of the breakpoints in a balanced t(1;13)(q21;q12) constitutional chromosome translocation in a patient with ganglioneuroblastoma.** *K. Sossey-Alaoui, G. Su, E. Kitamura, J.K. Cowell.* Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY.

Neuroblastoma (Nb) is a malignancy of the sympathetic nervous system which affects children in their first decade. It is the most common extra-cranial solid tumor in children with an incidence of approximately 1 in 8-10,000 live births annually and accounts for approximately 10% of all children's cancers. Despite even the most aggressive treatment regimens, Nb is still one of the hardest tumors to cure and accounts for 15% of all childhood deaths due to cancer. Ganglioneuroblastoma is a relatively benign form of Nb and consists of a mixture of fibrils, mature and maturing ganglion cells, as well as undifferentiated neuroblasts. During routine cytogenetic analysis of patients with different manifestations of neuroblastoma we have identified one patient with ganglioneuroblastoma that carries an apparently balanced t(1:13)(q21;q12) reciprocal translocation. Positional cloning of the translocation breakpoint on chromosome 13 resulted in the mapping of the breakpoint between coding exon 2 and exon 3 of the WAVE3 gene, a member of WASP gene family. Although the breakpoint region on chromosome 1 was localized to within 2 kb of genomic sequence, no gene was found to be interrupted on this chromosome. No fusion transcript was detected using RACE PCR and northern analyses. SSCP analysis was performed on 24 neuroblastoma tumors from different stages. Several nucleotide changes were detected in different tumors, most of which were either silent mutations or were found in the intronic sequence flanking the splice site junctions, and therefore were considered as polymorphisms rather than the disease causing mutations. The WAVE3 transcript is mainly expressed in the nervous system and, like all the members of the WASP gene family, WAVE3 is a key element in the actin polymerization and the cytoskelton organization, and therefore is important for cell differenciation and motility. We suggest that, because of its expression pattern and its function, WAVE3 is a candidate gene for tumor suppression activity, at least in some forms of neuroblastoma.

**PAX6 Functions in Suppression of Glioblastoms.** *Y.H. Zhou<sup>1</sup>, Y.X. Shi<sup>1</sup>, T. Glass<sup>1</sup>, J.B. Zheng<sup>2</sup>, G.F. Saunders<sup>2</sup>, W.K.A. Yung<sup>1</sup>.* 1) Dept Neuro-Oncology, MD Anderson Cancer Ctr, Houston, TX; 2) Dept Biochemistry and Molecular Biology, MD Anderson Cancer Ctr, Houston, TX.

Glioblastomas (GBMs) are the most common and malignant primary brain tumors. Approximately 6.0 cases per 100,000 person-year occur as GBMs, and an estimated 13,000 deaths in 2000 were attributed to such diseases. Pax6 gene encodes a transcription factor important for regulation of cell proliferation, migration and differentiation in the development of the eye and central nervous system, including the differentiation of radial glia in the cerebral cortex and the migration of glial precursors in the ventral neural tube of spinal cords. We found that in human glioma cell lines, PAX6 expressed at a lower level in high tumorigenic and at a higher level in low tumorigenic and nontumorigenic glioma cells. This encouraged us to investigate the possibility that PAX6 be involved in suppression of glioma progression. We examined the expression of the gene by real-time reverse transcription quantitative-PCR in a series of gliomas, including 42 GBMs and 34 anaplastic astrocytomas (AAs), and 6 normal brain tissues. The cDNA copy numbers of four house keeping genes (GAPDH, enolase-alpha, beta-actin and RPS9) were measured for each tumor cDNA samples. The relative expression levels of PAX6 were normalized by these four genes in combination after being weighted to a similar level. Significant difference ( $P < 0.001$ ) was observed between the expression of PAX6 in GBMs versus AAs. Furthermore, over expression of PAX6 in the high-tumorigenic human glioma cell U251HF through stable transfection of a PAX6-expression construct (PAX6/pRC-CMV) supports this hypothesis through demonstrating that PAX6 suppressed anchorage-independent growth, cell invasion by in vitro invasion assay, and the tumor growth in nude mice by subcutaneous tumor implantation. In conclusion, our data showed that the expression level of PAX6 could be used as a diagnostic marker for glioma progression. Studies to uncover the gene regulatory networks associated with the suppression function of PAX6 in glioma will provide clues in the cure of this most common and progressive brain cancer.

To be presented by Peter Nuernberg instead of K. Uhlmann.

Program Nr: 351

**Glioma subtypes show characteristic patterns of DNA hypomethylation and hypermethylation.** *K. Uhlmann<sup>1,2</sup>, K. Rohde<sup>2</sup>, C. Zeller<sup>1,2</sup>, J. Szymas<sup>3</sup>, S. Vogel<sup>4</sup>, K. Marczynek<sup>1</sup>, G. Thiel<sup>1</sup>, P. Nürnberg<sup>1,2</sup>, P.W. Laird<sup>5</sup>*. 1) Institute of Medical Genetics, Charité, Berlin, Germany; 2) Gene Mapping Center, MDC, Berlin-Buch, Germany; 3) Katedra Patomorfologii Klinicznej, Poznan, Poland; 4) Neurochirurgie, Sankt Gertrauden Krankenhaus, Berlin, Germany; 5) Department of Biochemistry and Molecular Biology, University of Southern California, Keck School of Medicine, Norris Comprehensive Cancer Center, Los Angeles, USA.

Gliomas are CNS tumors with a wide spectrum of different tumor types. They range from pilocytic astrocytomas, with a generally good prognosis, to the extremely aggressive malignant glioblastomas multiforme. In addition to these two types of contrasting neoplasms, several other subtypes can be distinguished, each characterized by typical phenotypic, as well as genotypic features. Recently, the epigenotype, as evident from differentially methylated DNA loci, has been proposed to be useful as a further criterion to distinguish between tumor types. In this study, we screened 139 tissue samples, including 33 pilocytic astrocytomas, 53 astrocytomas of different grades, 7 oligoastrocytomas, 10 oligodendrogliomas, 3 glioblastomas multiforme, and 33 control tissues for methylation at 15 different loci to generate epigenomic fingerprints. We used the semi-quantitative high-throughput method MethyLight to analyze a gene panel comprising *ARF*, *CDKN2B*, *RB1*, *APC*, *CDH1*, *ESR1*, *GSTP1*, *TGFBR2*, *THBS1*, *TIMP3*, *PTGS2*, *CTNNB1*, *CALCA*, *MYOD1*, and *HIC1*. Eight of these loci showed tumor-specific methylation changes. Within distinct subtypes, we found tissue- as well as grade-specific methylation patterns. Interestingly, pilocytic astrocytomas showed no evidence of CpG island hypermethylation, but rather were hypomethylated, relative to control tissues, at *EGFR*, *MYC*, *MYOD1*, and *TIMP3*. Our results show that (i) glioma subtypes have characteristic methylation patterns, (ii) grade and tissue of origin reveal distinct methylation patterns, (iii) hyper- as well as hypomethylation takes place in gliomas, and (iv) pilocytic astrocytomas are hypomethylated at several gene loci in comparison to control tissues.

**Glioma gene expression profiling and hierarchical clustering.** *R. Shai*<sup>1</sup>, *B. Merriman*<sup>1</sup>, *T. Kremen*<sup>2</sup>, *L. Liau*<sup>2</sup>, *P. Mischel*<sup>3</sup>, *T. Cloughesy*<sup>4</sup>, *S. Nelson*<sup>1</sup>. 1) Human Genetics, UCLA, Los Angeles, CA; 2) Neurosurgery, UCLA, Los Angeles, CA; 3) Pathology, UCLA, Los Angeles, CA; 4) Neurology, UCLA, Los Angeles, CA.

Gliomas are the most common form of brain tumors and are classified into several groups according to their malignancy and cell lineage. Accurate diagnosis of glioma type is fundamental for proper patient management. Currently the classification of the tumors is based on histopathology. While generally accurate and reproducible, the interpretation can be subjective with gray borders between groups. Further, little data exists to make individual specific prognosis. We used gene expression profiling and hierarchical clustering in order to classify tumors. First we tested the reproducibility of the gene expression based classification. Four samples of glioblastoma (GBM), two oligodendrogliomas and one normal brain sample were micro-dissected in several distinct areas of the tissue. Gene expression profiles of each region were generated using the Affymetrix 6800 gene chip. Clustering by similarity of expression profile revealed that multiple samples of the same tumor cluster similarly, indicating that there is not excessive heterogeneity in the expression profile throughout the tumors. Interestingly, cell lines derived from those tumors did not cluster with the parental tissues but clustered tightly together into a distinct group. Further gene expression analysis was performed on 21 *de novo* GBMs, 5 progressive GBMs, 3 oligodendrogliomas, 2 astrocytomas, and 2 low grade mixed gliomas with the U95A Affymetrix gene chip. The unstructured clustering of tissues generated a pattern, which predominantly grouped the tumors by histopathology. Genes that distinguished the GBMs from the normal tissue were selected to generate a list of 212 up regulated and 229 down regulated genes that had more than 5-fold change. Among these genes, several well-established tumor related genes can be found, for example *midkine* and *EGFR*. The identified differentially expressed genes also included ESTs and other genes whose involvement in tumorigenesis has to be assessed. Additional samples need to be analyzed for molecular prediction of prognosis, survival time and efficacy of treatments.

**Sporadic Colorectal Cancer Association with LOH-RER from Genetic Markers Application on Tumor Tissue --**

**- Bioinformatics Analysis.** *K.-H. Chen*<sup>1, 2, 3</sup>, *M.-H. Tsai*<sup>4</sup>, *Y.-C. Yang*<sup>5, 6</sup>. 1) Department of Laboratory Medicine, School of Medicine, National Taiwan University, Taipei, Taiwan; 2) Biostatistics Division, Institute of Epidemiology, School of Public Health, National Taiwan University, Taipei, Taiwan; 3) Department of Mathematics, School of Natural Sciences, National Taiwan University, Taipei, Taiwan; 4) Division of Colon and Rectal Surgery, Cardinal Tien Hospital, Taipei, Taiwan; 5) School of Medical Technology, College of Medicine, National Taiwan University, Taipei, Taiwan; 6) Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan.

Primary bioinformatics findings for AWD (alive with disease) verse DOD (dead of disease) associated with LOH and RER for 10 genetic markers on colorectal cancer tissues conclude that LOH of both APC and PT53\_5 is risk only when suitable promoted factors (PFs) have to co-exist, under a certain limiting environment (LE) in terms of LOH or RER, and possibly clinical/ pathology factors. LE of excluding only the binary outcome of metastasis to liver or not (MLON) allowed the clinical associations - the appearance of risk factors of LOH for both APC and PT53\_5 when the clinical information of tumor sites supplied; risk effect for LOH of APC when the stage of Dukes C or not supplied; and differentiation or not is protective. LE of additionally excluding LOH interaction (3-LOH) of PT53\_5, D8S254 and DCC, promoted gene (PG) of LOH-PT53\_5 promoted the risk effect of LOH-APC and the LOH interaction (PT53-LOH) of PT53\_2 and PT53\_5. Under LE of excluding MLON, 3-LOH and PT53-LOH in order, LOH of APC and PT53\_2 formed the risk factors when PG was only one of LOH-NM23, LOH-DCC, LOH-PT53\_2 itself, LOH-APC itself or RER-MSH2. Under LE of excluding MLON, 3-LOH, LOH-MSH2 and PT53-LOH in order, PG of interaction of LOH-PT53\_5 and RER-PT53\_2 promoted risk of LOH for APC and PT53\_2. The newly identified XLR (exact logistic regression) exclusively for binary information, under the multinomial distribution likelihood estimation, identified PGs and LEs and made the findings to be possible. The statistics nested by the LEs qualified from PGs have to be ascertained by Fisher's exact procedure.

**Identification of four homozygous deletions in human lung carcinomas in the 21q11.1-q21.1 region.** *C.J. Cox, G.R. Bignell, H.R. Molloy, P.J. Stephens, P.A. Futreal, R. Wooster, M.R. Stratton.* Cancer Genome Project, Sanger Centre, Cambridge, Cambridgeshire, UK.

Detection of frequent loss of heterozygosity (LOH) and homozygous deletions in human cancers are often good indicators of the presence of potential tumour suppressor genes. It has been reported previously that non-small cell lung carcinomas (NSCLC) show a high incidence of LOH on chromosome 21, relative to small cell lung carcinomas (SCLC) and other cancers. Here we report genotyping analysis of 616 cell lines (including 51 NSCLC, and 46 SCLC) with 5 polymorphic micro-satellite markers (density of 5.8Mb) specific for Chromosome 21. LOH was assigned to cell lines where 3 contiguous markers were found to be homozygous. LOH was found in 49% of NSCLC compared to 23% SCLC and 20% in all other cell lines. Eighty-eight cell lines showing LOH (including 21 NSCLC and 8 SCLC) were further screened with a set of 68 Chromosome 21 specific STSs (density of 0.48Mb), utilizing a high throughput form of Taqman analysis we have developed specific for CA repeats. This revealed the presence of homozygous deletions in 3 NSCLC and 1 SCLC between D21S1911 and D21S1905, overlapping a previously identified homozygous deletion found in a NSCLC.

A more detailed map of the deletions in this area was achieved using a further 68 STSs (density of 0.124Mb). This revealed that the deletions were made up of two smaller non-overlapping deletions (approximately 2.8 and 0.7Mb in size) encompassed by two larger overlapping deletions (approximately 6.2 and 6.5Mb in size). Primers for all coding exons of the 8 known genes in the region encompassed by the larger deletions have been designed and are currently being used to evaluate 92 cell lines with LOH on chromosome 21 for point mutations to identify the putative tumour suppressor gene(s).



**Genome wide analysis of 400 microsatellite repeats through 665 cancer cell lines.** *G.R. Bignell, C.J. Cox, H.R. Molloy, P.J. Stephens, P.A. Futreal, R. Wooster, M.R. Stratton.* Cancer Genome Project, The Sanger Centre, Hinxton Cambridge, UK.

Loss of heterozygosity is a key approach in identifying the genomic localisation of putative tumour suppressor genes. To identify new tumour suppressor genes, we have undertaken a genome wide analysis of a large number of cancer cell lines using a 400-marker map with an average spacing of 10cM. This analysis has been carried out on 665 cancer cell lines obtained from public repositories and private contributions, requiring a total of 266,000 genotypes, this represents the largest such screen carried out to date. We have a further 750 cell lines awaiting analysis. The current set included 125 lung, 65 breast, 42 colorectal, 36 leukaemia/lymphoma, 30 bladder, 31 melanoma and 26 ovarian cell lines. Because no parental DNA is available LOH has been provisionally assigned if five contiguous markers are shown to be homozygous. Data from this screen is being used to target chromosomal regions for large-scale mutation detection and for targeted homozygous deletion searches. In addition this dataset lends itself to a wide range of analysis, for instance comparison of percentage LOH for each chromosome arm through the complete set of cell lines or for specific tissue types, as well as being able to assess concomitant losses of particular chromosomal regions in particular tumour types. When comparing the levels of LOH for chromosomes 21 (14%) and 22 (22%) it can be seen that chromosome 22 has a higher rate of LOH even though these chromosomes are of similar size (Chr21 44.9Mb, Chr22 47.6Mb). This lends weight to the argument for a tumour suppressor gene located on chromosome 22. Patterns of loss for primary tumours in the literature can be confirmed against this dataset, for instance patterns of LOH in lung cancer for chromosomes 4q (average LOH from literature 42%, LOH from our data 51%) indicates the location of a putative tumour suppressor gene. Studies of this scale will help provide the basis for genome wide studies of cancer.

**Overexpression of cyclins B observed in human tumors alters chromosomal segregation.** *J.M. Flaman<sup>1</sup>, N. Sarafan-Vasseur<sup>1</sup>, J. Bourguignon<sup>1</sup>, F. Le Pessot<sup>2</sup>, A. Lamy<sup>1</sup>, R. Sesboué<sup>1</sup>, C. Bastard<sup>1</sup>, P. Hieter<sup>3</sup>, T. Frébourg<sup>1</sup>.* 1) INSERM EMI 9906, IFRMP, Faculté de Médecine, Rouen, France; 2) Anatomie et Cytologie Pathologiques, CHU de Rouen; 3) CMMT, Vancouver, Canada.

The molecular basis of chromosomal instability (CIN), which is observed in most of the malignant tumors, remains to be characterized. To identify genes which overexpression results in CIN, we developed a biological approach based on the use of a yeast indicator strain for CIN. In this strain, the *ade2-* mutation, resulting into the accumulation of a red pigment, has been suppressed by an ochre suppressing tRNA, carried by a non essential artificial chromosomal fragment, and this strain is therefore spontaneously white. Induction of CIN in this strain will result into the loss, during the mitosis, of the chromosomal fragment and therefore into the appearance of red sectors within the white colonies. Screening into this CIN indicator strain of a yeast genomic library led us to identify, among the clones generating 100% of sectoring colonies, *Clb5*, one of six B-type cyclins identified in yeast. Overexpression of cyclin B2 and cyclin B1, the two human homologs of *Clb5*, into the CIN indicator strain resulted also into a sectoring phenotype and induced, like overexpression of *Clb5*, an abnormal sensitivity to benomyl, indicating that overexpression of B type cyclins alters the spindle checkpoint. Using multiplex real-time quantitative RT-PCR, we analyzed cyclins B1 and B2 mRNA expression in a serie of 58 primary colorectal cancers. In 10% of the tumors, we observed a 10 fold increase of cyclin B2 mRNA, in comparison to normal colorectal mucosa. Although we detected no overexpression of cyclin B1 mRNA, immunohistochemical staining revealed a high level of cyclin B1 expression in 12 % of the tumors. These results show that overexpression of cyclins B is observed in human cancers and could contribute, through an alteration of the spindle checkpoint, to chromosomal instability.

**Paternal uniparental disomy 11p15, hemihyperplasia and hepatoblastoma.** *L. Celle<sup>1</sup>, K.L. Russell<sup>1</sup>, D.J. Zand<sup>1</sup>, A.T. Meadows<sup>2</sup>, J. Pressey<sup>2</sup>, D. von Allmen<sup>3</sup>, R. Weksberg<sup>4</sup>, E.H. Zackai<sup>1</sup>.* 1) Division of Human Genetics and Molecular Biology; 2) Division of Oncology; 3) Department of Pediatric General and Thoracic Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA; 4) Department of Genetics, Hospital for Sick Children, Toronto, Canada.

Hepatoblastoma is a rare embryonal tumor occurring most frequently before the age of 3 years. Patients with Beckwith-Wiedemann syndrome (BWS), isolated hemihyperplasia and the combination of both have an increased risk of neoplasia with Wilms' tumor being the most common, followed by adrenocortical carcinoma and hepatoblastoma. Patients with BWS and some with isolated hemihyperplasia have been shown to have deregulation of normal imprinted expression on chromosome 11p15. Here we present a child with hemihyperplasia with paternal UPD 11p15 who, after tumor surveillance, was found to have an elevated AFP and subsequent diagnosis of hepatoblastoma. A 4.5-month old boy was referred to Genetics for hemihyperplasia. He was a 9lb 1oz product of a FT pregnancy without neonatal hypoglycemia. On physical exam, there was marked hemihyperplasia of the right arm and leg, with a 1cm difference in the length of the humeri and tibiae. There was also an enlargement of the right side of the tongue. There was no umbilical defect and no abnormal ear creases. Molecular genetic studies showed paternal UPD of chromosome 11p15. He was followed with abdominal ultrasounds and alpha-feto protein (AFP) levels. At 5months, the AFP level was noted to be 325.8ng/ml (normal 0.6-28.3), fell during the next several months, and rose again at 10months. Although the ultrasound at that time was normal, a CT scan and MRI with gadolinium enhancement revealed a 2 x 1.7cm lesion in the right lobe of the liver. Following complete excision, pathology revealed hepatoblastoma, mixed histology. This report emphasizes that children with hemihyperplasia should undergo molecular testing. They should also have careful tumor surveillance, especially those with paternal UPD 11p15.

**Chromosomal amplifications and deletions determined by comparative genomic hybridization in follicular thyroid adenomas and carcinomas - similar patterns of chromosomal aberrations in a subset of thyroid**

**carcinomas.** *D.J. Marsh<sup>1</sup>, G. Theodosopoulos<sup>1</sup>, L. Delbridge<sup>2</sup>, B.G. Robinson<sup>1,3</sup>.* 1) Kolling Institute of Medical Research and University of Sydney, NSW, Australia; 2) Department of Surgery, Royal North Shore Hospital, NSW, Australia; 3) Department of Medicine, University of Sydney, NSW, Australia.

Carcinoma of the thyroid is the most frequently diagnosed endocrine malignancy. Malignant lesions of the follicular cells are comprised of the differentiated tumors follicular thyroid carcinoma (FTC) and papillary thyroid carcinoma, whilst anaplastic thyroid carcinoma is an undifferentiated malignancy. Follicular thyroid adenomas (FTAs) are benign tumors. We used comparative genomic hybridization (CGH) to elucidate regions of chromosomal gains and losses in both FTAs and FTCs. Seventy-six % (13 of 17) of FTCs showed CGH changes, however only 24 % (5 of 21) of FTAs showed changes. The number of CGH changes varied markedly, with FTAs showing between 1 - 8 (mean = 3.6) CGH changes, and FTCs showing 3 - 23 (mean = 11.5). Of the changes observed in FTAs, none were observed more than once. However, certain "hot-spots" were evident in the FTCs including gain of chromosomes 7, 5 and 12, as well as loss of chromosomes 1, 8 and 11. A number of chromosomal alterations were found in both FTAs and FTCs, including loss of 3p, 4, 8, 9p and X, as well as gains of 4q, 5p, 7, 8, 9, 12 and 17. Gain of 1p and loss of 5q were unique to FTAs. Of considerable interest, 6 of the FTCs had between 14 - 23 (mean = 20) CGH changes, and these changes appeared to be largely consistent between the tumors, including loss of chromosome 1, 2, 3, 6, 8, 11 and X, as well as gains of chromosomes 5, 7, 12, 19 and 22. This data is suggestive that activation of a mutator phenotype occurs at some point in a progression pathway during thyroid tumorigenesis and in at least a subset of tumors, leads to a non-random pattern of chromosomal gains and losses. Alternative patterns of chromosomal gains and losses may not be conducive to survival of the malignant cell. It is possible that such a mutator phenotype involves the alteration of a gene(s) functioning to regulate mitosis.

**Over-Expression of the TSPY Gene on the Y Chromosome Potentiates Cell Proliferation in vitro and in vivo. X.X. Liu, Y.F.C. Lau.** Department of Medicine, University of California, San Francisco, CA.

The testis-specific protein Y-encoded (TSPY) gene is a repeated gene on the human Y chromosome. It encodes a protein homologous to a family of cyclin B binding proteins and is postulated to serve a normal function in directing the spermatogonial cells to enter meiosis in the testis. Based on its chromosomal location, it has been considered to be a candidate for the gonadoblastoma (GBY) locus on the Y chromosome. Expression studies indeed demonstrated a high level expression of this GBY candidate gene in gonadoblastoma and testicular seminoma. We hypothesized that aberrant expression of TSPY in cells incapable of entering male meiosis is responsible for its oncogenic activities. To test this hypothesis, we used the tetracycline (Tet-off) regulation system to manipulate the expression of transfected TSPY gene in HeLa or NIH3T3 cells. Cells over-expressing TSPY exhibited a 30-45% higher proliferative activities than those under repressed conditions or transfected with the vector alone. This increase in proliferative activities paralleled with an elevated rate of colony formation in transfected cell population cultured in normal media than that cultured in media containing doxycycline. Subcutaneous inoculation of stably transfected HeLa cells in immunodeficient nude mice showed a faster rate of tumor formation in hosts fed with normal drinking water than that in hosts fed with doxycycline-containing water. Significantly, NIH3T3 cells over-expressing the stably transfected TSPY gene formed tumors in nude mice fed with normal drinking water. However, the same transformant cells inoculated in hosts fed with doxycycline-containing water did not produce any tumor. Results from this study support the hypothesis that over-expression of the Y-located TSPY gene potentiates cell proliferation in vitro and in vivo, thereby signifying TSPY as a putative proto-oncogene on the human Y chromosome.

**Loss of heterozygosity of the CUTL1 gene in uterine leiomyomata.** *S.M. Zeng, B.J. Van Voorhis, J. Yankowitz.* Dept of OB/GYN, Univ Iowa Hosps & Clinics, Iowa City, IA.

Uterine leiomyomata or fibroids are common benign smooth muscle tumors. These tumors are the single largest cause of hysterectomy in the United States, accounting for approximately one in three hysterectomies or 200,000 procedures a year in the United States. The underlying cause of fibroids is still obscure. Recent molecular genetic and cytogenetic evidence indicates several gross and microscopic genetic alterations involving five main subgroups; del(7q), 6p rearrangements, del(13q), t(12,14), and trisomy 12. The gross and microscopic deletions at 7q may involve the human Cut-like homeobox gene, CUTL1, located at 7q22. We investigated loss of heterozygosity (LOH) of this gene in patients with uterine leiomyoma.

Samples of uterine fibroids and grossly normal myometrium were obtained from women having myomectomies or hysterectomies. Tissues were digested and DNA extracted by standard techniques. DNA samples were amplified using different sets of primers specific for three microsatellite markers inside CUTL1, D7S515, D7S518 and D7S666. PCR products were run on a 6% denaturing polyacrylamide gel. LOH was evaluated by comparing allele bands between tumor and normal uterine tissue from the same patient.

Twenty patients with both fibroid tissue and normal surrounding tissue were evaluated. A CUTL1 deletion was found in three fibroid samples (15%). In one of these patients LOH was also present in one of two "normal" surrounding tissue samples. In one patient with LOH in one large fibroid another small fibroid did not show LOH for the CUTL1 markers.

Our results agree with previous findings from cytogenetic and molecular genetic studies supporting the hypothesis that CUTL1 gene may be a tumor suppressor and whose deletion or loss may play a role in the pathogenesis of uterine leiomyoma. Additional studies are needed to elucidate the relationship between DNA alterations, potential other factors, and fibroid development.

**Identification of a minimal overlapping amplified region at 19q13.1-q13.2 in four ovarian cancer cell lines.** *T.C.M. Tang, J.S.T. Sham, X.Y. Guan.* Department of Clinical Oncology, The University of Hong Kong, Hong Kong, People's Republic of China.

High-level amplification of DNA sequence at 19q13.1-q13.2 in ovarian cancer has been frequently detected by using chromosome microdissection and comparative genomic hybridization (CGH). This strongly suggests that 19q13.1-q13.2 contains a putative oncogene(s) which plays an important role in the development or progression of ovarian cancer. In order to narrow down the amplified region at 19q13.1-q13.2 (about 39cM), four ovarian cancer cell lines which have been confirmed containing amplicon at 19q13.1-q13.2 were studied for identifying a minimal overlapping amplified region (MAR). Fluorescence in situ hybridization (FISH) with cosmid clones and Southern blot analysis with cDNA probes in average genomic distance at 1 cM (from D19S425 to D19S418) were performed. At least two MARs have been identified at 19q13.12 (about 200 kb) and 19q13.13 (about 1.2 Mb), respectively. Amplification and overexpression of several candidate oncogenes within the two amplicons have been studied by Southern and Northern blot analysis in four ovarian cancer cell lines. Further study of genes within these two amplicons may lead to the isolation of the oncogene(s) which is the biological target of amplification events in ovarian cancer.

***HER2/neu* and *c-MYC* oncogene alterations in breast lesions from women with *BRCA1/2* gene mutations.** C.L. Soderberg, K.A. Anderl, C. Adem, C. Reynolds, J.M. Slezak, L.C. Hartmann, R.B. Jenkins. Mayo Clinic, Rochester, MN.

**Introduction:** Oncogene amplification is frequently observed in breast carcinogenesis. *HER2/neu* and *c-myc* are overexpressed/amplified in 25-30% and 5-50% of sporadic breast malignancies, respectively. Breast cancers arising in patients with *BRCA1* and *BRCA2* germline mutations have specific clinicopathologic features. Previous authors have reported lower levels of overexpression of *HER2/neu* protein among *BRCA1* mutation carriers. Thus, progression pathways might differ in hereditary versus sporadic cases. As part of our investigation of the pathogenesis of *BRCA1/2* related cancers, we are evaluating multiple genetic alterations in neoplastic and preneoplastic lesions from women with and without a family history of breast cancer. **Materials and Methods:** Patients with a family history of breast cancer and germline *BRCA1/2* mutations who underwent therapeutic and prophylactic mastectomy (TM, PM) were selected as cases. Patients without a family history of breast cancer and without *BRCA1/2* mutations who underwent TM and PM, matched by age and surgery date, were selected as controls. All available paraffin blocks with neoplastic and precursor lesions were evaluated by fluorescent in situ hybridization (FISH) using *HER2/neu/CEN17* and *cMYC/CEN8* dual-probe mixtures. **Results:** Currently, we have studied precursor and neoplastic lesions in 16 hereditary cases (out of 29) and 19 sporadic cases (out of 40). *BRCA1/2* related cancers had a higher incidence of amplifications/gains compared to matched controls in the in situ (50% vs 36% for *HER2/neu*, and 83% vs 45% for *cMYC*), and invasive components (57% vs 37.5% for *HER2/neu*, and 72% vs 44% for *cMYC*). All but one precursor lesion had no apparent anomalies. **Conclusion:** Our data suggest that chromosomal alterations, as measured by CEN8 and CEN17 gain and *HER2/neu* and *cMYC* gene amplification, are more frequent within breast cancers of women with a strong family history of cancer, consistent with an increased underlying chromosomal instability. Ongoing studies are directed toward the elucidation of the pathways of carcinogenesis in hereditary versus sporadic disease.



**A common founder for the V126D CDKN2A mutation in seven North American melanoma-prone families.** *A.M. Goldstein<sup>1</sup>, L. Liu<sup>2</sup>, M.G. Shennan<sup>3</sup>, D. Hogg<sup>2,4</sup>, M.A. Tucker<sup>1</sup>, J.P. Struewing<sup>1</sup>.* 1) Division of Cancer Epidemiology and Genetics, National Cancer Inst, Bethesda, MD; 2) Department of Medicine, University of Toronto, Toronto, ON; 3) Division of Medical Oncology, Toronto-Sunnybrook Regional Cancer Centre, Toronto, ON; 4) Department of Medical Biophysics, University of Toronto, Toronto, ON.

Germ-line mutations in the major known melanoma (MM) susceptibility gene CDKN2A have been observed in approximately 20 percent of melanoma-prone families. One of the most common melanoma-related CDKN2A mutations reported in North America is the V126D mutation. We examined nine markers (IFNA-D9S736-D9S1749-[CDKN2A]-D9S974- D9S942- D9S1748-D9S1604-D9S171-D9S126) surrounding CDKN2A in three American and four Canadian families carrying the V126D mutation to determine whether the mutation resulted from a single origin and to date the origination of the mutation. The Canadian families had an average of 3 MM patients per family; two families had patients with multiple primary MM. The American families had an average of 7 MM patients per family; all had patients with multiple primary MM. Six of the seven families had German/English ancestries. All seven families had a haplotype consistent with a common ancestor/founder for the V126D mutation. Using maximum likelihood methods, we estimated the mutation to have originated 34-52 generations ago (1-LOD-unit support interval 13-98 generations). Most recurrent CDKN2A mutations observed in North America can be traced back to a European country or region of origin. In contrast, the V126D mutation does not appear at high frequency in any other countries besides the United States and Canada. This phenomenon may reflect selective mutation testing or it may be related to the origination of the V126D mutation. Although six of the seven studied families immigrated to North America from Germany and England, the ancestral pathway for melanoma in these families cannot be determined. Additional families from North America and other geographic areas may help determine the geographic origin for this recurrent yet puzzling CDKN2A mutation.

**Identification of novel regions of allelic imbalance (AI) in young onset colorectal cancer (CRC) with normal mismatch repair (MMR).** *L.A. Boardman<sup>1</sup>, S.J. Schmidt,<sup>2</sup> G.M. Petersen<sup>3</sup>, N.M. Lindor<sup>4</sup>, H. Nelson<sup>6</sup>, R.M.*

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Between 7000 and 20,000 individuals under age 50 will develop CRC in the U.S. in 2001. Up to one third of these cases can be explained by known genetic syndromes, but the cause of the majority of young onset CRC remains unknown. Discovery of genes responsible for CRC using AI have focused on older onset CRC. We hypothesize that AI can inform gene discovery in young onset CRC. We identified a sample of 47 cases of non-FAP young onset CRC with normal DNA mismatch repair (MMR), by immunohistochemistry for hMLH1 and hMSH2 and microsatellite instability. We used two comparison groups: 100 older onset non FAP, MMR CRC tumors tested for AI and previously published reports of AI in sporadic CRC. Genome wide AI was assessed at 25 to 50 cM intervals with 65 markers. AI was assessed as positive if the ratio difference between normal and tumor allele intensity was  $> 2$ . AI was detected in  $> 20\%$  of the young onset tumors on five chromosomal arms: 5q; 15q, 17p 18q and 20p. Although AI at 8p has been reported in 50%; of CRCs tested, in our study, 8p AI occurred in  $< 20\%$  of young onset CRC. Both young and older onset CRC demonstrated similar levels of AI on chromosomal arms 5q, 17p, and 18q, corresponding to loci for the tumor suppressor genes, APC, p53 and DCC (SMAD4), respectively. AI on chromosomal arms 15q and 20p, rarely detected in the older onset group, was present in over 20% of young onset CRC with normal MMR. These results suggest that the genetic events that contribute to the development of young onset CRC may differ from those in older onset CRC. Further finer mapping with higher density AI may uncover other genetic loci leading to young onset CRC with normal MMR.

**A novel homozygous mutation in the human *MSH2* gene predisposes to leukemia and multiple cafe-au-lait spots.**

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Individuals with a germline mutation in one of the DNA mismatch repair (MMR) genes are at significant risk for colorectal cancer and are also susceptible to developing tumours of the endometrium, colon, urinary tract and other tumours of the gastrointestinal system. Two families have previously been reported with individuals homozygous for mutations in the MMR gene *MLH1* that are predicted to compromise MMR. These individuals develop haematological malignancies and neurofibromatosis type 1 (NF1) at an early age. Here we demonstrate a patient where a homozygous novel mutation in another MMR gene, *MSH2*, is associated with leukemia and multiple cafe-au-lait spots, a feature of NF1. The mutation occurs within an invariant splice site consensus sequence and is predicted to result in a truncated non-functional protein. Therefore, it is the general lack of mismatch repair from conception that appears to underlie the haematological malignancies and occurrence of sporadic NF1 in these families. The haematological malignancies observed in the individuals homozygous for the loss of MMR are reflective of the T and B cell lymphomas seen in homozygous mice lacking MMR and suggest that the MMR 'knock-out' mice may provide a useful model for human neoplasia. The effect of the lack of MMR on genetic instability, particularly the NF1 gene in this patient, will be discussed.

**A regular screening regimen decreases mortality in von-Hippel Lindau families.** *J.S. Green, C. Moores, S. Murphy, G. Wong, P. Parfrey.* Depts of Medical Genetics and Medicine, Memorial Univ of Newfoundland, St John's, NF., Canada.

Von-Hippel Lindau (VHL) Syndrome is an autosomal dominant condition with almost complete penetrance. Affected individuals have increased risk of vascular tumours including retinal angioma (RA), cerebellar (CH) and spinal cord (SPH) hemangioblastoma, pheochromocytoma (PH), and renal cell carcinoma (RCC), as well as cysts and adenomas of the pancreas, kidney, and other abdominal organs. There is an early age at onset (often in childhood) and affected individuals often have multiple different manifestations. The first Newfoundland (NF) VHL family was ascertained in 1982, at which point a screening protocol was designed and initiated for NF VHL families (beginning at 5 years of age). A total of 5 NF VHL families (each with a different VHL mutation) have since been identified, with a total of 131 (64 M, 67 F) affected and 50%-risk individuals. Records of screening investigations and treatment were reviewed for all family members. Within the largest family (N=49 affected) the most common tumour observed was PH (71%), followed by RA (61%), CH (51%), and RCC (31%). Genetic testing was offered to all at 50% risk and there was a high uptake of testing. The total cohort (N=131) included 64 mutation positive (59 clinically affected), 50 mutation negative, and 17 individuals of unknown mutation status. Kaplan-Meier life table analysis was used to calculate cumulative age to death in screened and unscreened groups. A mean survival time of 43.6 years (95%CI:39.3-47.8), with 62% mortality by age 50 was found for affected individuals not followed in a screening program, with the most common causes of death being PH and CH. Mortality was decreased in the 40 individuals who entered the screening program, showing a mean survival time of 56.6 years (95%CI:51.9-61.3) and 21% mortality by age 50. Only one death occurred in the 35 individuals who entered screening before age 30. Mean survival time for the mutation negative group was 76.5 years (95%CI:70.0-84.1), with 11% mortality by age 50. Screening of at-risk individuals can identify tumours at an early stage when many can be successfully treated, reducing mortality in VHL families.

**Different combinations of biallelic APC mutations confer different growth advantages in colorectal tumours.** *J.P. Cheadle, M. Krawczak, M.W. Thomas, A.K. Hodges, N. Al-Tassan, N. Fleming, J.R. Sampson.* Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, UK.

Biallelic mutations of the APC gene are very early events in the development of sporadic and familial adenomatous polyposis (FAP)-associated colorectal tumours in humans. Recently, several new facets to Knudson's '2-hit' hypothesis have been proposed in relation to APC: protein inactivation may be selected weakly, if at all, and the 'two hits' may be interdependent. We carried out a review of all published data on colorectal adenomas and carcinomas with characterised somatic APC mutations and identified reports on 165 sporadic and 102 FAP-associated tumours with two defined mutations. Using a Poisson model for the observed number of mutations per amino acid residue, we redefined the mutation cluster region (MCR) to residues 1281-1556, and demonstrated that the locations of the two APC mutations, relative to the MCR, are not independent ( $p < 0.0001$  in both sporadic and FAP-associated tumours). We developed a model based upon the data for sporadic tumours which suggests that different growth advantages are being conferred by different combinations of APC mutations. Thus, genotype I/I (I: truncating mutation inside MCR) is 3.9 times more likely to be selected than genotype IO or IL (O: truncating mutation outside MCR, L: allelic loss), which in turn is 27.8 times more likely to be selected than OO or OL. The data for FAP-associated tumours did not fit the same model, which could in principle reflect different mechanisms of sporadic and FAP-associated tumourigenesis, but may also result from biased FAP patient sampling and mutation screening.

**Effect of altitude on the clinical severity of hereditary paraganglioma.** *K. Astrom<sup>1</sup>, J.E. Willett-Brozick<sup>2</sup>, E.N. Myers<sup>3</sup>, B.E. Baysal<sup>1,2,3</sup>.* 1) Hum Genet, Univ. Pittsburgh,; 2) Dept Psychiatry, Univ. Pittsburgh Med. Ctr; 3) Dept. Otolaryngology Univ. Pittsburgh Med. Ctr., Pittsburgh, PA.

Hereditary paraganglioma (PGL) is characterized by the development of benign, vascularized tumors in the head and neck. The most common tumor site is the carotid body (CB), a highly vascularized small organ that senses blood oxygen levels. PGL is primarily caused by mutations in the SDHD gene (PGL1) of the mitochondrial complex II. The SDHD mutations in PGL are likely to act by loss of function of the protein product. However, there is significant amount of phenotypic variability among the PGL patients that needs to be explained. Chronic hypoxic stimulation of high altitudes induces neoplastic growth in the CB and the incidence of sporadic paraganglioma tumors increases in proportion to the altitude. There is a marked phenotypic similarity between the CB tumors in PGL and those that develop in high altitudes. This suggests that the availability of environmental oxygen may be an important factor in determining the clinical severity of the PGL tumors. To test this hypothesis, the data on the clinical severity of the tumors in PGL patients are tabulated based on what altitude the patients lived until the first tumors were recognized. The severity of the tumors is assessed by scoring the age-of-onset, the number of tumors, tumor size and metastasis. Questionnaires have been sent to over 140 patients belonging to more than 40 PGL families who have been recruited to the PGL research study at the University of Pittsburgh. Preliminary data suggests that individuals living in higher altitudes have more severe phenotypes. For example, rare metastatic PGL tumors have been observed in two individuals in a single PGL1 family who lives in an altitude above 2000 feet. In contrast, the identification of founder mutations in almost 40% of "sporadic" patients in the Netherlands suggests a mild clinical phenotype in a location situated in very low altitudes. These preliminary findings have important implications for the management of PGL patients and suggest that the phenotypic expression of the genetic defects in PGL may be influenced by the availability of environmental oxygen.

**The Finnish R726L androgen receptor mutation is uncommon in prostate cancer families in the United States.**

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The observation that prostate cancer aggregates in families has led investigators to search for germline mutations that may increase the susceptibility to prostate cancer. Since the relative risk attributed to having a brother with prostate cancer is twice as high as that conferred by an affected father, some investigators have suggested that there may be X-linked and/or recessive prostate cancer loci. The androgen receptor (AR) gene (OMIM \*313700), located at Xq11-12, has been considered to be a candidate prostate cancer susceptibility gene. Recently, Mononen et al. (*Cancer Res*, 60: 6479, 2000) reported that a germline AR R726L mutation was detected in 2% of Finnish men with sporadic or familial prostate cancer and was associated with an approximately six-fold increased risk of prostate cancer. The University of Michigan Prostate Cancer Genetics Project is a family-based study dedicated toward characterizing the molecular basis of prostate cancer. Enrollment criteria for his study include early-onset and/or a family history of prostate cancer. Five hundred forty eight men with histologically confirmed prostate cancer from 411 unrelated families were selected for this study. The average age of prostate cancer diagnosis was 61.0 +/- 9.6 years. ASO hybridization was used to detect the presence of this mutation in germline DNA extracted from lymphocytes. A Finnish DNA sample containing the R726L mutation was used as a positive control for all experiments. None of the 548 prostate cancer patients studied, including 513 White, 29 African American, 3 Asian and 3 Hispanic men, were found to carry the AR R726L allele. Therefore, the prevalence of this allele is significantly less than that observed among Finnish men with prostate cancer (Fisher's exact test,  $p = 0.002$ ). Our data is consistent with the hypothesis that the AR R726L allele is a founder prostate cancer allele in Finland, and that this mutation does not account for a significant proportion of early-onset and/or familial prostate cancer in the United States.

**Mutation and methylation analysis of the *PTEN* gene in a population-based series of endometrial carcinoma patients.** *S. Das*<sup>1</sup>, *E.I. Kretzschmar*<sup>1</sup>, *P. Gruber*<sup>1</sup>, *L.A. Akslen*<sup>2</sup>, *H.B. Salvesen*<sup>2,3</sup>. 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Department of Pathology, The Gade Institute, Bergen, Norway; 3) Department of Gynecology and Obstetrics, Haukeland University Hospital, Bergen, Norway.

Mutations of the *PTEN* gene are a frequent finding in endometrial carcinoma. Promoter hypermethylation, an alternative mechanism of gene inactivation, has more recently been observed in the *PTEN* gene in endometrial carcinoma. In an effort to understand the different mechanisms of *PTEN* inactivation in endometrial carcinoma and the relationship between the different mechanisms, we are studying a series of endometrial carcinoma tumors for *PTEN* mutation, methylation and loss of expression. We have available for our studies a well characterized population-based series of endometrial carcinomas with complete follow-up. We have performed mutation analysis of the *PTEN* gene in 145 endometrial carcinoma tumors by denaturing high performance liquid chromatography (DHPLC) followed by sequence analysis. To date we have found mutations in approximately 45% of our tumor set. The mutations observed are all predicted to be deleterious and include insertion, deletion, nonsense and non-conservative missense mutations. 24% of mutations were found in regions of repetitive sequence. Approximately 14% of the tumors studied so far have two different mutations in the *PTEN* gene. The *PTEN* methylation status of this tumor set has previously been determined and found to occur in approximately 20% of tumors. We are in the process of combining our mutation and methylation results to determine the overall mechanism of *PTEN* inactivation in these tumors. In addition, we are studying these tumors for *PTEN* expression and will determine how tumors with *PTEN* mutation and/or methylation correlate to expression.



**A Protease Resistant Form of DNA Ligase III Protects HT1080 Cells From Programmed Cell Death.** *L. Bordone, C. Campbell.* Department of Pharmacology Medical School, University of Minnesota, Minneapolis, MN.

A yeast two-hybrid screen identified the calcium-dependent protease calpain as a putative DNA ligase III-binding protein. Consistent with this finding, in vitro transcribed and translated recombinant DNA ligase III protein was a substrate for calpain degradation. Addition of calpain to nuclear extracts resulted in degradation of endogenous DNA ligase III. This calpain-mediated proteolysis was calcium dependent and was blocked by the specific calpain inhibitor calpeptin. Experiments revealed that calpain binds to the N-terminal region of DNA ligase III. This portion of DNA ligase III contains an acidic domain (named PEST) that is frequently present in proteins cleaved by calpain. A mutant version of DNA ligase III protein that lacked a PEST sequence did not bind to calpain in a yeast two-hybrid system. In addition, this mutant version of DNA ligase III was resistant to in vitro proteolysis by calpain. Since a variety of DNA repair enzymes are degraded during apoptosis, we hypothesized that DNA ligase III proteolysis is essential for cell death. We therefore isolated HT1080 clones that overexpressed either mutant or wild type forms of DNA ligase III. Cytotoxicity experiments were performed by treating these cells with ionizing radiation, the topoisomerase inhibitor I camptothecin, or anti-Fas antibody. Cells expressing the DNA ligase III clone that lacked the PEST sequence were more resistant to killing by all three of these agents than were cells that expressed the wild-type form of DNA ligase III. These results suggest that DNA ligase III cleavage is an essential step in programmed cell death in human cells.

**High prevalence of multiple melanoma in patients with CDKN2A mutations from melanoma-prone families. C.**

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Cyclin-dependent kinase inhibitor 2A (CDKN2A) is considered the main candidate gene for melanoma susceptibility and in 1993 Petty et al. described a patient affected from multiple melanoma with a constitutional deletion of this region. Recently CDKN2A mutations were described also in other solid tumors as brain gliomas, bladder, esophagus, breast and pancreas carcinomas. We collected 56 melanoma-prone families and analyzed the three coding exons and the 5' UTR of CDKN2A gene. For each patient we elaborated a three generation pedigree in order to evidence any other subject affected from melanoma or other different tumors and consider the presence of multiple melanomas. We identified a CDKN2A mutation in 8 out of 56 families (14%), 5 already described in literature and three novel mutations. In particular we found three missense mutations (Gly101Trp, Arg24Pro, Pro48Thr), one small deletion (del201C) that produces a stop codon (145) by frameshift, and a splicing mutation (ivs1+ 2(T-C)). We think relevant that 6 out of 8 subjects were affected by multiple melanoma while among the 48 subjects without CDKN2A mutation only 2 showed a multiple melanoma. This evidence allowed us to consider the presence of a CDKN2A mutation in a patient affected by multiple melanoma as a marker sufficient to suggest the extension of the analysis to all the healthy members of the family. In fact, considering the high prevalence of the multiple melanomas in our families with CDKN2A mutations, supported by literature, we have to consider the possibility of a high neoplastic risk ( melanoma, pancreas or larinx carcinoma) associated to the presence of CDKN2A mutation.

**A predictive model of metachronous colorectal carcinoma in HNPCC.** *L. Bertario<sup>1</sup>, A. Russo<sup>2</sup>, P. Sala<sup>1</sup>, A. Viel<sup>3</sup>, M. Genuardi<sup>4</sup>, M. Ponz de Leon<sup>5</sup>, A. Cama<sup>6</sup>, C. Mareni<sup>7</sup>, P. Radice<sup>1</sup>.* 1) National Cancer Institute, Milano; 2) Local Health Authority, Milano; 3) Centro Riferimento Oncologico, Aviano; 4) Cattolica University, Rome; 5) University of Modena; 6) University of Chieti; 7) University of Genoa - Italy.

HNPCC-related colorectal carcinoma (CRC) cases show a tendency to the development of both synchronous and metachronous lesions. The identification of risk factors for multiple cancers might contribute to define the appropriate clinical treatment in these patients. We carried out a retrospective analysis of 479 CRC cases from HNPCC families fulfilling the Amsterdam Criteria I or II, recruited by six collaborating Italian institutions. Approximately 62% were early stages (Dukes A-B) and 38% advanced stages (Dukes C-D). The anatomical distribution was as follows: proximal colon in 222 cases (46%), distal colon in 108 cases (23%), rectum in 84 cases (18%), multiple locations in 32 cases (7%). A total of 203 patients (42%) belonged to families in which a germline mutations, either in the MLH1 (n = 126) or MSH2 gene (n = 77), could be identified. Metachronous CRCs developed in 49 patients, with a mean interval from the first cancer of 78 months (range 4-288 months), and a cumulative incidence at 10 years of 18.7%. The identification of the best predictive model for metachronous CRCs was carried using a training/testing technique. The largest collection of cases from a single center, that of the National Cancer Institute including 227 patients, was used as a training set. In this group, a multivariate logistic regression analysis revealed that early stage (OR 10.1), distal location of primary CRC (OR 2.9), and MLH1 mutations (OR 4.2) were associated with an increased risk for metachronous carcinoma. A logistic model derived from these observations was subsequently evaluated on a testing test composed of 252 patients collected by the other participating centers, in which 67% of metachronous cancers were correctly predicted. These findings indicate that integrating information on primary cancer stage and location and on mutational status of HNPCC individuals with CRCs may predict those at higher risk for metachronous lesions.

**CDKN2A germline mutation analysis in patients with pancreatic adenocarcinoma from Liguria.** *P. Ghiorzo<sup>1</sup>, L. Pastorino<sup>1</sup>, A.M. Nicora<sup>2</sup>, L. Bonelli<sup>2</sup>, S. Zupo<sup>1,2</sup>, B. Villaggio<sup>3</sup>, M.R. Sertoli<sup>1,2</sup>, G. Bianchi-Scarrà<sup>1</sup>, V. Pugliese<sup>1,2</sup>.* 1) Oncology Biol & Genetics, Univ Genova; 2) National Institute for Cancer Research, Genova; 3) Department of Internal Medicine, Univ Genova, Italy.

Pancreatic adenocarcinoma is one of the leading causes of cancer deaths worldwide. In some families showing aggregation of PC, malignant melanoma and breast cancer, germline mutations in the p16 tsg have been identified in populations where a founder effect is prevalent. It has been estimated that 5-10% of PC are associated with strong familial predisposition but the proportion of pancreatic cancer attributable to inherited mutations of CDKN2A remains largely unknown. The present study aims to investigate the contribution of CDKN2A mutations in unselected pancreatic cancer patients, consecutively diagnosed in Liguria, where the G101W mutation is recurrent in melanoma families which are at increased risk of developing other cancers. Between April 1, 1998 and March 31, 2001, 59 unrelated patients (33 males and 27 females, mean age at diagnosis 64.9 years, range 41-83 years) were referred to ERCP and were diagnosed with exocrine pancreatic cancer at IST in Genoa. A detailed oncologic pedigree was obtained from 55 subjects. DNA-analysis was performed in 30 consenting patients (13 males and 17 females, mean age at diagnosis 66.7 years, range 48-83 years). The neoplasm was located in the head of the pancreas in 21 cases (77.4%). A histologic diagnosis was available for 11 cases. According to pedigree information 8 of 30 pts (26.7%) were considered as belonging to cancer-prone kindreds (2 of them were diagnosed with both MM and PC). Patients in this sub-set were slightly younger than sporadic patients (mean age 62.9 year $\pm$ 11.0 vs 68.0 years $\pm$ 10.3), (P=0.25). Screening of CDKN2A showed germline CDKN2A mutations in 2/30 unselected pancreatic patients. However, these 2 mutations were identified among the 8 patients belonging to cancer-prone families. These results suggest that the occurrence of PC in multi-cancer families in Liguria may be linked to underlying genetic susceptibility due to recurrent CDKN2A germline mutations. This study was supported by Ministry of Health grants N.ICS 0701/RF 99.63 to MRS and GBS.

**Association between the occurrence of breast cancer and cartilaginous tumors: phenotypic characterization of a hitherto unrecognized potential hereditary trait.** *A.M. Cleton-Jansen<sup>1,2</sup>, M. Timmerman<sup>1</sup>, M.J. Van de Vijver<sup>4</sup>, C.J. Van Asperen<sup>3</sup>, L.C.J.M. Van den Broek<sup>1</sup>, P.C.W. Hogendoorn<sup>1</sup>.* 1) Pathology, LUMC, Leiden, The Netherlands; 2) Human and Clinical Genetics, LUMC, Leiden, The Netherlands; 3) Clinical Genetics, LUMC, Leiden, The Netherlands; 4) Dutch Cancer Institute, Amsterdam, The Netherlands.

Recently we documented a strong association between the occurrence of cartilaginous tumors (enchondroma, chondrosarcoma) and breast cancer in the same patient, using a nation-wide case-control study. This study revealed an odds ratio of 7.62 for a potential association of breast and cartilaginous tumors, pointing statistically strongly towards a genetic trait. This is furthermore corroborated by the age of onset in patients with breast cancer as the first tumor, which is about 10 years earlier than breast cancer in the general population. The Dutch BRCA1 and BRCA2 family database was searched, however no case of chondrosarcoma nor enchondroma was reported, neither within the same patient nor within the pedigree pointing to a trait which is different from the afore mentioned breast cancer syndromes. Following this statistical/epidemiological analysis we report on the phenotypic characterization of the patient group. Using the national pathology database the tissue blocks of all patients reported to fulfil the associated tumors mentioned were retrieved. Reported diagnoses were reviewed; breast cancer specimens were classified and histologically characterized according to the procedures used by the Breast Cancer Linkage Consortium. In addition the cartilaginous tumors were analyzed with emphasis on the central versus peripheral localization in the skeleton as previous studies proved a different molecular mechanism to be operative in the different subtypes. Remarkably all cartilaginous tumors are of one common histological subtype being centrally localized whereas no peripheral cartilaginous tumor was registered. The breast tumors were histologically heterogeneous with varying differentiation grade. Results on immunohistochemical staining (p53, Bcl2, neu, p16, p21 estrogen and progesterone receptor and E-cadherin) will be presented.

**Androgen Receptor CAG repeats length in Jewish Israeli women who are BRCA1/2 mutation carriers: relevance to breast/ovarian cancer morbidity.** *E. Dagan*<sup>1,2</sup>, *E. Friedman*<sup>3</sup>, *T. Paperna*<sup>1</sup>, *N. Carmi*<sup>3</sup>, *R. Gershoni-Baruch*<sup>1,4</sup>. 1) Dept Human Genetics, Rambam Medical Ctr, Haifa, Israel; 2) Dept Nursing, faculty of Welfare and Health Sciences, University of Haifa, Israel; 3) Susanne Levy Gertner Oncogenetic Unit, Sheba Medical Center, Tel Aviv, Israel; 4) Technion-Institute of Technology, Rappaport Medical School, Haifa, Israel.

BRCA1/2 mutation carriers are at an increased lifetime risk for developing breast and/or ovarian cancer. The factors that govern the phenotypic expression of mutant BRCA1/2 alleles have not been fully elucidated. The polymorphic CAG repeats within the Androgen Receptor (AR) gene which encode for polyglutamine tract may interact with BRCA1/2 via steroid hormone pathway regulation, and hence is a candidate BRCA modifier gene. Previous studies have shown that the CAG repeat size may be associated with BRCA1 mutation morbidity. To extend this observation, we genotyped 227 women, carriers of one of the predominant Jewish BRCA1/2 mutation, for the AR CAG repeats, and evaluated the contribution of this polymorphism to breast/ovarian cancer morbidity. Of 227 BRCA1/2 carriers, 169 were BRCA1 mutation carriers (185delAG, n=130; Tyr978X, n=2 and 5382insC, n=37) and 58 carried the 6174delT BRCA2 mutation. Unilateral breast cancer was diagnosed in 79 women, bilateral breast cancer in 15, ovarian cancer in 41, breast/ovarian cancer in 14 and 78 were asymptomatic mutation carriers. Mean age at diagnosis among affected women (with either or both neoplasms) 46.71±1.2 years and that of the asymptomatic group - 45.89±1.4 did not differ. The AR CAG repeats ranged from 11 to 28. None had an allele longer than 29 CAG repeats. The mean number of CAG repeats was not statistically different between the asymptomatic group (203; range, 14-28) and the patients with either early onset breast cancer (<42 years at diagnosis) (193; range, 11-26) or with both breast and ovarian cancer (193; range, 14-28). Variation in CAG repeats length was not correlated with age at diagnosis or clinical manifestation. This study does not support the notion that AR CAG repeats contribute to the phenotypic expression of mutant BRCA allele in Jewish individuals.

**Comparative analysis of methods for detecting BRCA1 rearrangements in breast-ovarian cancer families. B.**

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Recent studies have identified BRCA1 rearrangements in about 10% of families with a high probability of genetic predisposition to breast and ovarian cancer and negative for BRCA1/2 coding-regions mutations. To study such families, our group compared four approaches : DNA combing, cDNA analysis of size variation and of loss of expressed allelic markers, gene dosage by real time quantitative PCR and semi-quantitative fluorescent multiplex PCR. Our goals were : (1) to compare sensitivity and specificity of these methods in the detection of BRCA1 rearrangements in routine laboratory settings (2) to estimate the contribution of BRCA1 (and subsequently BRCA2) germ-line rearrangements in French breast and ovarian cancer families. Known deletions/duplications were used to validate the performance of the three methods that rely on genomic DNA analysis. These methods were applied initially to 13 families with negative BRCA1/2 mutation test results. Family eligibility criteria were two or more cases of ovarian cancer in 1st degree relatives (2d degree if transmission was by men) or at least one case with both breast and ovarian cancer. DNA combing and cDNA analysis required carefully isolated and frozen lymphocytes or lymphoblastoid cell lines which currently limits their use for retrospective analysis of samples. Conversely, both gene dosage assays have the potential for screening large numbers of families with high sensitivity and specificity and have been selected for the second phase of our study in a larger series of French patients.

**Multiple colorectal adenomas in familial colorectal cancer: more evidence for disease diversity and genetic heterogeneity.** *K.S. Dahan<sup>1</sup>, M. Smaers<sup>1</sup>, X. Pepermans<sup>1</sup>, C. Sempoux<sup>2</sup>, P. Van Maele<sup>3</sup>, A. Kartheuser<sup>4</sup>, Ch. Verellen-Dumoulin<sup>1</sup>.* 1) Dept Genetics, UCL Saint-Luc Hosp, Brussels, Belgium; 2) Dept of Pathology, UCL, Brussels; 3) St-Jean Hospital, Brussels; 4) Dept of Surgery, UCL, Brussels.

Twenty to 30 % of colon cancers are associated with a familial risk manifested as an early-onset tumours or multiple lesions of several sites. There is evidence that low-penetrance missense variants (I1307K and E1317Q) and mutations located within the 5' and 3' regions of APC gene predispose to multiple colorectal adenomas whereas germline mutations in mismatch repair (MMR) genes are characterised by a family history of early-onset cancers of specific sites. The aim is to find out what proportion of multiple adenomas patients have disease resulting from APC or MMR genes. **Patients and methods** : we have studied adenoma specimens of 22 Belgian unrelated patients with a familial risk of cancers and 3 to 25 colorectal adenomas for microsatellite instability (MSI) and expression of MLH1 and MSH2 proteins. Germline mutation was carried out by DGGE and direct sequencing throughout the APC gene and the *MLH1* and *MSH2* genes for MSI (+) individuals. **Results**: MSI is detected in 9 adenomas (9/22) with no expression of MLH1 (1/9), of MSH2 (2/9) and with a focal expression of MSH2 (2/9). In the 4 last cases, a normal expression of the two MMR products is observed. No germline mutation of *MLH1* and *MSH2* is detected. Out of 22 individuals, only one truncating APC variant in exon 9 (R332X) is identified. **Conclusion**: Among patients with multiple adenomas and familial history of colorectal cancer, the disease seems to be correlated to a MMR deficiency due to the absence of MSH2 in 18 % whereas germline alterations of APC gene are rare in our studied group. Furthermore, to understand the implication of MMR in the genesis of multiple adenomas , a screening of an another candidate gene such *MSH6* interacting with MSH2 protein should be useful in patients with MSH2(-)adenomas.



**Uptake of Prophylactic Surgery and Screening Compliance in Hereditary Breast and Ovarian Cancer.** *J.N.*

*Everett<sup>1</sup>, M. Pritzlaff<sup>2</sup>, C. Christianson<sup>1</sup>, J. Groden<sup>3</sup>, K. Huelsman<sup>1</sup>.* 1) Human Genetics, Children's Hospital Medical Center, Cincinnati, OH; 2) University of Cincinnati, College of Allied Health Sciences, Cincinnati, OH; 3) University of Cincinnati, College of Medicine, Cincinnati, OH.

**Purpose:** To examine compliance with screening recommendations as well as interest in and uptake of prophylactic surgery after genetic counseling among women clinically evaluated for hereditary breast and ovarian cancer risk.

**Patients and methods:** Questionnaires were sent to 264 women who received genetic counseling through the Hereditary Cancer Program, Cincinnati, OH. 145 women completed the self-administered questionnaire (54.9% response rate). Responses were compared to data collected at the time of clinical evaluation in order to determine changes in screening behaviors since genetic counseling.

**Results:** Women who tested positive for BRCA1 or BRCA2 mutations showed significant improvement in breast cancer screening practices. These women were also more likely to have had or be considering prophylactic mastectomy ( $p=0.0025$ ) and prophylactic oophorectomy ( $p<0.001$ ) than were women who tested negative or did not have testing. Improvements in ovarian cancer screening after counseling were not observed in this study, regardless of mutation status. Only 55% of women over age 50 were compliant with general population guidelines for colon cancer screening.

**Conclusions:** Genetic counseling for hereditary breast and ovarian cancer in the clinic setting is associated with improvements in breast cancer screening, particularly among women testing positive for BRCA1 and BRCA2 mutations. Genetic counseling and testing also appears to have an impact on decision-making with regard to prophylactic mastectomy and prophylactic oophorectomy. Limitations of the ovarian screening may partially explain lack of improvement in compliance with this screening. Colon screening recommendations should be discussed during genetic counseling in conjunction with other cancer screening recommendations.

**FAP and HNPCC mutations in the same patient.** *C.D. DeLozier-Blanchet<sup>1</sup>, E. Arrigoni<sup>2</sup>, Z. Dobbie<sup>3</sup>, C. Rey-Berthod<sup>4</sup>, M-A. Brundler<sup>5</sup>, J-L. Blouin<sup>1</sup>, C. Soravia<sup>6</sup>, P. Hutter<sup>4</sup>.* 1) Div Medical Genetics, Geneva Univ Hospital; 2) Div Gastroenterology, Geneva Univ Hospital; 3) Human Genetics, University Hospital Basel; 4) Unit of Genetics, ICHV, Sion; 5) Div Clinical Pathology, Geneva Univ Hospital; 6) Digestive Surgery Clinic, Geneva University Hospital, SWITZERLAND.

Colonoscopy was performed in a healthy 24 year-old Italian man whose father and multiple relatives reportedly had polyposis coli. His mother was one of nine healthy sibs. Microsatellite analysis (MSI) of an excised dysplastic adenoma vs. lymphocyte DNA revealed high-level instability (MSI-H); immunohistochemistry showed absence of hMSH2 and hMSH6 proteins in the adenomas. An insertion mutation was detected in exon 7 of hMSH2 (ins1191G), leading to protein truncation 19 codons downstream. On prophylactic colectomy at age 25 a small adenocarcinoma (T1N0M0) and multiple flat adenomas and associated aberrant crypts were revealed. The father, sure of his own diagnosis of FAP, was then available; he did not have the hMSH2 mutation, but PTT revealed an truncating exon 15 mutation in the APC gene (del3471-3474GAGA). The proband thus has both a hMSH2 mutation of unknown origin and a paternally-inherited APC mutation. Gastroscopy revealed multiple glandulo-kystic polyps in the gastric fundus, but none in the duodenum. Endoscopy of upper and lower digestive tracts has been recommended every 6 months. The risk that potential offspring inherit one or both cancer-predisposing genes is 75%. We are aware of one other patient (J Med Genet 1999;36:790-93) with mutations in both the APC and a HNPCC gene: a missense mutation in exon 12 of hMSH2 and the I1307K missense variant in APC, documented in the Ashkenasi Jewish population and considered a pre-mutation.

**BRCA1 and BRCA2 Genotyping of Cases Ascertained in Year One of the Population-Based Ontario Familial Breast Cancer Registry.** *G.D. Glendon<sup>1</sup>, H. Ozcelik<sup>4,2</sup>, J.A. Knight<sup>2</sup>, N. Carson<sup>7</sup>, P. Ainsworth<sup>5</sup>, S. Taylor<sup>6</sup>, R.*

*Carter<sup>8</sup>, N.F. Boyd<sup>3</sup>, I.L. Andrulis and the Ontario Cancer Genetics Network<sup>1,2</sup>.* 1) Cancer Care Ontario, Toronto, ON, CA; 2) Samuel Lunenfeld Res. Inst., Mount Sinai Hospital, Toronto, ON, CA; 3) Ontario Cancer Institute, Princess Margaret Hospital, Toronto, ON, CA; 4) Dept. Path. and Lab. Medicine, Mount Sinai Hospital, Toronto, ON, CA; 5) Mol. Diag. Lab., London Health Sciences Centre, London, ON, CA; 6) Dept. of Path., Queens University, Kingston, ON, CA; 7) Mol. Gen. Diag. Lab., Children's Hospital of Eastern Ontario, Ottawa, ON, CA; 8) Dept. of Path. and Mol. Med., Chedoke-McMaster Hospital, McMaster University, Hamilton, ON, CA.

The OFBCR is a population-based registry that collects family and personal history information, as well as blood samples, from incident breast cancer cases and their relatives throughout Ontario for use in studies of familial breast cancer. It is one of the six sites that comprise the Cooperative Family Registry for Breast Cancer Studies. Incident invasive breast cancer cases diagnosed between Jan. 1996 and Dec. 1998 in all women aged 20-54 and 35% of those between 55-69 and all men aged 20-79 were ascertained via the Ontario Cancer Registry. Those that met additional personal or family history criteria were designated as familial and were eligible for protein truncation analysis with 5' DNA sequencing for deleterious mutations in the BRCA1/2 genes. This report is limited to probands diagnosed in 1996. Of the 315 familial cases analysed, 31 (9.8%) were found to carry BRCA mutations (17 in BRCA1 and 14 in BRCA2). As expected, multivariate analysis determined that the presence of early onset breast and ovarian cancer in either the proband ( $p=0.006$ ) or relatives ( $p=0.006$ ) was a good predictor of a BRCA1 mutation. Both the presence of multiple primary breast cancer in the proband ( $p=0.003$ ) and at least 2 second degree relatives with breast cancer ( $p=0.032$ ) were good predictors of BRCA2 mutations. As well, Ashkenazi Jewish ancestry ( $p=0.003$ ) was a good predictor of either a BRCA1 or 2 mutation. Analysis with a full 3-year ascertainment will allow us to more fully explore the predictors of harbouring a BRCA mutation.

**Follow-up molecular studies of prostate cancer families.** *D.S. Gerhard<sup>1,2</sup>, B.K. Suarez<sup>2,1</sup>, L.T. Nguyen<sup>1</sup>, M.E. Arthur<sup>1</sup>, J. Lin<sup>2</sup>, B. Haberer<sup>3</sup>, P. Humphrey<sup>4</sup>, S. Finkelstein<sup>5</sup>, O. Schirripa<sup>6</sup>, W.J. Catalona<sup>3</sup>.* 1) Dept. of Genetics, Box 8232; 2) Dept. of Psychiatry; 3) Dept. of Urology; 4) Dept. of Pathology, Washington University School of Medicine, St. Louis, MO; 5) Dept. of Pathology, University of Pittsburgh; 6) Dept. of Pathology, University of Colorado Health Sciences Center, Denver.

Prostate cancer (CaP) is the most common cancer in American men. At least 9% of the cases are due to a genetic predisposition though the mode of inheritance is unclear as autosomal dominant, recessive, X-linked or multigenic transmission was reported. In the 3 genome screens of CaP families published to date, positive findings include 3 loci on chromosome 1, at least 1 locus on chromosome X and at least 1 locus on chromosome 16. However, none of these genes have been identified, therefore, to clone at least one of the genes we followed up the results of our genome screen in 2 ways; one, testing of potential candidate genes by association, and two, characterization of the tumors for deletions.

We tested the androgen receptor (AR), in our sample of ~260 multiplex affected kindreds and 355 controls. AR is a transcription factor upon binding of androgens. It has 2 polymorphic sites within exon 1 (the transactivation domain), CAG and GGC. It has been suggested that the shorter form of the protein has increased function and may be correlated with increased frequency in CaP patients. In our sample, we did not find any association between the length of the CAG repeat and presence of CaP. The analysis of the GGC as well as of the haplotype is in progress. In our genome screen, the marker with the highest linkage signal was D16S3096. We are genotyping available tumor DNAs (not all patients underwent surgery) with markers between and including D16S3040 and D16S515. Here we report the analysis of the first 125 tumor DNAs that amplified; 64 tumors (51%) have a deletion of at least one chromosome 16 marker, though unexpectedly, not all tumors have a common region of deletion. We plan to analyze the tumors by their linked or unlinked to chromosome 16 status and determine if the deletion patterns are associated with the linkage results.

**Good response to chemotherapy (CT) and hormonotherapy (HT) in patients with *BRCA1*-related breast cancer (*BRCA1*-BC).** P.O. Chappuis<sup>1,2</sup>, J. Goffin<sup>1</sup>, N. Hamel<sup>2</sup>, N. Wong<sup>1</sup>, A.J. Paradis<sup>1</sup>, D. Roberge<sup>1</sup>, J.S. Brunet<sup>3</sup>, C. Yee<sup>4</sup>, P. Tonin<sup>2</sup>, J. Boyd<sup>4</sup>, P. Ghadirian<sup>5</sup>, L.R. Bégin<sup>1,6</sup>, W.D. Foulkes<sup>1,2</sup>. 1) SMBD-Jewish and Montreal General Hospitals, Montreal, QC, Canada; 2) Research Institute, McGill University Health Center, Montreal, QC, Canada; 3) Algorithme Pharma Inc., Montreal, QC, Canada; 4) Memorial Sloan Kettering Cancer Centre, New York, NY; 5) CHUM-Hôtel-Dieu, University of Montreal, QC, Canada; 6) Sacré-Coeur Hospital, Montreal, QC, Canada.

The prognosis of *BRCA1*-BC may be worse than that of sporadic BC. We studied the response to CT and/or HT of *BRCA1*-BC in 2 different groups of BC patients. A) We determined the impact of adjuvant CT and HT in a historical cohort of 292 women diagnosed with BC < age 65 at one hospital between 1980-95. All women were Ashkenazi Jewish and were tested for the 3 founder mutations in *BRCA1/2*. Follow-up (f/u) was available for 275 (94.2%) patients. Data were censored at 8 years (median f/u). B) We reviewed the charts of all known *BRCA1* mutation carriers identified in Montreal who received neo-adjuvant CT. A) Thirty (10.9%) *BRCA1* mutations were identified. At the median f/u, *BRCA1* mutation carriers had a significantly worse overall survival (OS) compared with non-carriers ( $P=0.016$ ). Among patients who do not receive adjuvant CT or HT, OS was significantly worse among *BRCA1* mutation carriers compared with non-carriers ( $P=0.003$  and  $P=0.011$ , respectively). No difference of OS was noted among patients who received CT or HT (both  $P > 0.45$ ). B) 5 *BRCA1* mutation carriers received pre-operative adriamycin-based CT for primary BC. Despite a relatively large tumor size (median: 6cm) and 2 cases clinically N+, a complete clinical response was noted in 4/5 cases and a complete pathological response in 3/4. One case showed no significant PR, another could not be pathologically evaluated. Patients with *BRCA1*-BC have a similar outcome compared to non-carriers only if they received adjuvant treatment. This could be crucial for small, N- tumors where the indication for adjuvant treatment might otherwise be questionable. *BRCA1* mutation carriers showed a surprisingly good response rate to neo-adjuvant CT.

**Association of the 15-kDa selenoprotein gene (*SEP15*) with malignant mesothelioma.** *S. Apostolou, Y. Mitsuuchi, J.R. Testa.* Human Genetics Program, Fox Chase Cancer Ctr, Philadelphia, PA.

Exposure to asbestos is a risk factor for malignant mesothelioma (MM) development, although little is known about the molecular pathogenesis of this cancer. Suppression subtractive hybridization was used to identify genes down regulated in MM cell lines compared to normal mesothelial cells. *SEP15* was isolated using this technique and was subsequently demonstrated to be down regulated in ~50% of MM cell lines. This gene encodes a selenium containing protein and maps to 1p31. Since loss of heterozygosity (LOH) is frequently exhibited at 1p31 in MM, and the trace element selenium is a candidate chemopreventive agent in several cancers, we decided to investigate the possible role of *SEP15* in MM.

SSCP and RFLP analyses of *SEP15* revealed a polymorphism at position 1125 in the 3'UTR SECIS recognition element. This element is necessary for decoding in-frame TGA codons as selenocysteine, rather than terminating translation. The association between polymorphism 1125G/A in 27 MM cases and 48 population controls was investigated. A significantly increased frequency of the 1125A variant was found in MM cases versus controls ( $P=0.04$ ), using the Chi-square test.

Since this variant lies within the SECIS element, we analyzed the effects of selenium on the growth of normal mesothelial cells and MM cell lines possessing wild type *SEP15* or the 1125A variant. We also analyzed MM cell lines in which *SEP15* was down regulated. Dose-dependent growth inhibition was observed in the cancer cells; however, cells with 1125A were found to be less responsive to added selenium than cells with wild type protein. Moreover, cells with down regulation of *SEP15* displayed the least growth inhibitory response to selenium.

Thus, individuals with the 1125A polymorphism may have increased risk of developing MM and may be less responsive to the protective benefits of dietary selenium. The findings of this study, together with frequent LOH at the *SEP15* locus, suggest that *SEP15* may be involved in the development of MM and/or the mechanism by which selenium functions in cancer prevention.

**Genetic counseling guidelines for families of children with acute leukemia.** *I.M. Kedar-Barnes, S.E Plon.* Baylor College of Medicine, Houston, TX.

Acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML) are the most common childhood cancers yet little is known about the genetics of these disorders and guidelines for genetic counseling referral have not been developed. Most children with ALL/AML do not have a genetic predisposition for developing leukemia and in 1% of the cases there is some familial clustering. Genetic syndromes that predispose to leukemia include: Bloom syndrome, Fanconi anemia, ataxia telangiectasia, neurofibromatosis type I, Li-Fraumeni syndrome, inherited immunodeficiency syndromes and chromosomal aneuploidy including Down syndrome, Turner syndrome and Klinefelter. For these cases recurrence risk is specific to that syndrome. For "familial" ALL/AML counseling is a challenge and consists of education and understanding of recurrence risk until leukemia predisposition genes have been better identified. Siblings of children with isolated ALL/AML have about a 2-4 fold relative risk, however their absolute risk remains low. Recurrence risk is increased when there are multiple family members with hematologic cancers. Examples of such cases are included: Case 1: A 3-yr. old girl diagnosed with ALL had an aunt with leukemia at age 5. The family has an increased risk approximately 3-5 fold over that of a single affected. Case 2: A 6-yr. old boy, one of identical twins had ALL and the parents were concerned for the twin. Recurrence risk in identical twins is increased but is age dependent and is approximately 20% for twins at that age. Case 3: A 22-yr. old woman had ALL at age 12. Her concerns were focused on her future offspring and their recurrence risk. Studies have shown no increased risk of cancer in offspring of cancer survivors, however, there is still a slight increased risk due to mutagen exposure during treatment. In summary, appropriate referrals for genetic evaluation are: families with a genetic disorder known to predispose to ALL/AML; family history of other cancers in multiple relatives suggestive of Li-Fraumeni syndrome; a family history of ALL/AML in 1st or 2nd degree relatives; identical twins (< age 6) with one affected twin with ALL and finally, long term survivors of childhood ALL/AML who reach reproductive age.

**A Population-Based Survey of Colorectal Cancer Screening in HNPCC Families.** *L. Madlensky<sup>1</sup>, V. Goel<sup>2</sup>.* 1) Institute of Medical Science; 2) Dept. of Health Administration, University of Toronto, Canada.

**Objective:** To determine whether at-risk members of putative HNPCC kindreds are undergoing colonoscopic or other screening for colorectal cancer (CRC), and to compare family histories and demographics of screeners and non-screeners.

**Methods:** Relatives of CRC cases registered in a population-based familial CRC registry in Ontario, Canada were surveyed about their history of fecal occult blood testing (FOBT), sigmoidoscopy and colonoscopy. Data were collected on the types of tests, reasons for tests, demographics, and family history of CRC.

**Results:** The survey sample represents high-risk family members accrued through the proband, rather than through a high-risk clinic or genetic counselling service. Most of the respondents had not yet had genetic counselling and/or testing at the time of the survey. There were 183 respondents from putative HNPCC kindreds (3 or more 1st/2nd degree relatives with CRC) over age 35. Forty (22%) had not had any of the three tests, 56 (31%) had one type of test (37 were colonoscopy), and 85 (47%) had >1 type of test (70 include colonoscopy). Eighty respondents reported having an FOBT (64% for screening purposes), 52 had a sigmoidoscopy (46% for screening) and 115 had a colonoscopy (72% for screening). Ninety-four (51%) of the respondents could be clearly classified as "screeners", and 41 (22%) as "non-screeners"; the remainder were considered "diagnostic" or "unclear". Screeners were significantly older than non-screeners (58 yrs vs. 50 yrs,  $p=0.003$ ), but there was no significant difference in sex, marital status, education or income. The mean number of CRC-affected relatives did not differ significantly between the two groups (4.7 vs. 4.2).

**Conclusions:** While the majority of HNPCC family members are screened by colonoscopy (the recommended modality), 37% of this increased risk group have not had a colonoscopy, and 22% have not had any type of screening test. This points to a need to develop or improve counselling and educational interventions that could increase the uptake of colonoscopic screening, which has been shown to improve CRC outcomes in HNPCC families.



**Constitutive Missense *MSH6* Mutations Associated with HNPCC and Familial non-HNPCC Colon Cancer**

**Patients.** A.E. Lagarde<sup>1,2</sup>, E. Baumann<sup>1</sup>, S. Viertelhausen<sup>3</sup>, H.S. Stern<sup>1,2,3</sup>. 1) Ottawa Health Research Institute; 2) Department of Surgery, University of Ottawa; 3) Ottawa Regional Cancer Center, Ottawa, Ontario, Canada.

Germline mutations in the *MSH2* and *MLH1* DNA-mismatch repair genes are detected in 50% of HNPCC families fulfilling the Amsterdam criteria and in less than 10% of suspected HNPCC cases. Functionally inactivated *MSH2* or *MLH1* confers microsatellite instability (MSI+). *MSH6* is also part of the DNA-repair protein complex. However, the role of the *MSH6* gene in susceptibility to cancer remains uncertain. Its involvement in HNPCC and other types of familial cancers has been presented. Yet, bi-allelic *MSH6* inactivation occurs inconsistently, and tumor MSI may vary greatly. We analyzed the *MSH6* gene in a group of  $n=31$  colorectal cancer patients with known family histories and MSI tumor phenotypes, in whom *MSH2* and *MLH1* mutations had been excluded. Direct sequencing of ten PCR products covering the entire *MSH6* coding region revealed two constitutive mutations, providing a 6.5% frequency estimate (95% CI= 0.8-24). There was no association with high-(0/4 cases) or low-MSI (0/17 cases). *Val878Ala* and *Ile872Val* are missense mutations located in exon 4. Both were found in the subset of ten MSI-negative tumors. The *Val878Ala* carrier was diagnosed with rectal cancer at the age of 50, and was a member of a typical, early-onset HNPCC family. A single *MSH6* carrier was identified among all five HNPCC and five suspected HNPCC patients. The novel, *Ile872Val* mutant allele was not detected among 92 control DNAs. It was found in a 72-year old patient who reported only one affected sibling. A single *MSH6* carrier was identified among all 18 such familial non-HNPCC cases (frequency: 5.6%; 95% CI= 0.14- 27). Analysis of tumor DNAs revealed no (0/31 cases) change in the (C)<sub>6</sub> microsatellite, in exon 5, and no other alterations in the *MSH6* gene of the two carriers. Our findings extend the notion that mutations in *MSH6* contribute a significant proportion of familial colorectal cancer cases, inclusive of, but not limited to HNPCC, which do not express the severe hallmarks (MSI) of other DNA-mismatch repair gene deficiencies.

**Mammographic density in *BRCA1/2* mutation-carriers.** *G. Mitchell*<sup>1</sup>, *R. Warren*<sup>2</sup>, *D. Easton*<sup>3</sup>, *R.A. Eeles*<sup>1</sup>. 1) Section of Cancer Genetics, Inst CA Res, Sutton, Surrey, England; 2) Dept of Radiology, Addenbrooks Hospital, Cambridge, UK; 3) CRC Genetic Epidemiology Unit, Strangeways Laboratory, Cambridge, UK.

The positive association between increasing mammographic density and increasing breast cancer risk has been extensively reported for the general female population. On small report has suggested that the breasts of *BRCA1* mutation-carriers are denser than age-matched controls with implications for mammographic screening sensitivity. **AIM** To determine whether mammographic density of known *BRCA1/2* mutation-carriers and non-carriers can predict mutation status or be useful for breast cancer risk stratification in carriers. **METHODS** Mammograms of *BRCA1/2* mutation-carriers and women with a negative predictive test (negs) at our institution were collected. Mammographic density was scored using the Wolff and Boyd classification systems. **RESULTS** 71 *BRCA* mutation-carriers were identified, 47 previously affected by breast cancer (pos aff). 42 were negs. 78% of mammograms were located, 72% of pos aff, 88% of unaffected carriers (pos unaff), 79% of negs. Odds ratios (adjusted for age) were calculated for comparisons of density between all categories and the least dense category for both systems.  $X^2$  test for trend with increasing density for all carriers VS negs was 2.84 ( $p=0.09$ ). For other comparisons, pos aff VS negs or pos unaff VS negs, carriers tended to have the most dense mammographic category (non significant). ORs for weight, menopausal status and hormone use is still in progress. **CONCLUSIONS** An interesting non-significant trend to increased mammographic density in mutation-carriers was observed. A larger study is planned to confirm these observations. Funding generously provided by Breast Cancer Campaign.

**Ovarian Cancer Incidence in a Screened Population of Jewish Women.** A. Liede<sup>1</sup>, B. Karlan<sup>2</sup>, R.L. Baldwin<sup>2</sup>, K. Glover<sup>2</sup>, L. Platt<sup>2</sup>, G. Kuperstein<sup>2</sup>, S. Narod<sup>1</sup>. 1) Centre for Research in Women's Health, Univ Toronto, Toronto, ON, Canada; 2) Gilda Radner Ovarian Cancer Detection Program, Cedars-Sinai Medical Center, UCLA, Los Angeles, CA.

Risk factors for ovarian cancer include a family history of ovarian cancer, Ashkenazi Jewish ethnicity and the presence of an inherited mutation in BRCA1 or BRCA2. The Gilda Radner Ovarian Cancer Detection Program in Los Angeles was established in 1991 to study the efficacy of screening in the early detection of ovarian cancer in women at risk for ovarian cancer. We studied a historical cohort of 291 Jewish women who participated in the Gilda Radner Program. Each of these women had a family history of ovarian cancer, or of early-onset breast cancer and received biannual ultrasound and serum marker evaluations including CA 125. The women were offered BRCA testing for three common founder mutations in the Jewish population (BRCA1 185delAG, 5382insC and BRCA2 6174delT). Seventeen incident cases of cancer were observed in the cohort from 1991 to 2000 (1,120 per 100,000 per year) including eight ovarian/peritoneal/tubal cancers and six breast cancers. The majority (86%) of women with incident breast or ovarian cancer carried a mutation in the BRCA1 gene. The overall cancer incidence among carriers of mutations in the BRCA1 gene was estimated to be 5,510 per 100,000 per year, corresponding to a cumulative incidence of 47.5% at ten years (2.5% at ten years for non-carriers  $P < 10^{-8}$ ). After adjustment of sampling, risks to female carriers of germline BRCA1 mutations at ten years were estimated at 21% for ovarian/peritoneal/tubal cancer, 16% for breast cancer, and 36% for any cancer. Most of the ovarian cancers presented in symptomatic women who had evidence of cancer spread beyond the ovary. The excess risk of breast and ovarian cancer in Jewish women with a family history of ovarian cancer is largely attributable to mutations in BRCA1. Intensive surveillance using CA 125 and ultrasound does not appear to be an effective means of diagnosing stage I epithelial ovarian cancer in this high risk cohort.

**Glutathione S-transferase theta and mu (GSTM1 and GSTT1) gene expression in patients with aplastic anemia and myelodysplastic syndrome.** *C.J. Osgood<sup>1,2</sup>, A.C. Spivey<sup>1</sup>, M.W. Stacey<sup>2</sup>, A.H. Kim<sup>2</sup>.* 1) Dept Biological Sci, Old Dominion Univ, Norfolk, VA; 2) Dept of Pediatrics, Eastern Virginia Medical School, Norfolk, VA.

Aplastic anemia (AA) and myelodysplastic syndrome (MDS) are disorders of the hemopoietic bone marrow cells that are frequently associated with drug and environmental exposures. Treatment-induced MDS can follow chemotherapy. An individual's inability to detoxify ingested chemicals may increase risk for disease. Glutathione S-transferases are Phase II drug metabolizing enzymes and play key roles in detoxification. GSTM1 and GSTT1 are members of this multigene family and both exist as polymorphic deletion variants within human populations. Individuals deficient in one or both genes may be at increased risk for development of AA or MDS. The frequency of genotypic deletion may underestimate the true proportion of individuals who are phenotypically non-expressors. To test whether the null phenotype is higher than the null genotype, patients non-null for GSTM1 and or GSTT1 (or both) were tested for expression using RT-PCR. Of 9 patients who were GSTM1 positive, 1 failed to show expression. Of 36 patients who were GSTT1 positive, 5 showed no expression. In 15 patients with both genes, 2 showed no expression. In all, 20% of tested patients were phenotypic nulls. DNA methylation within the GST gene promoter regions does not appear to explain these results.

**Characterization of high-risk colon cancers in the Utah population.** *D.W. Neklason<sup>1,2</sup>, W.S. Samowitz<sup>3</sup>, R.A. Kerber<sup>1,2</sup>, G.P. Mineau<sup>1,2</sup>, R.W. Burt<sup>2,4</sup>.* 1) Dept. Oncological Sciences; 2) Huntsman Cancer Institute; 3) Dept. Surgical Pathology; 4) Dept. Medicine, Univ. Utah, Salt Lake City, UT.

A small but undetermined fraction of familial cases arise from the colon cancer syndromes of familial adenomatous polyposis (FAP), attenuated FAP (AFAP) and hereditary nonpolyposis colon cancer (HNPCC). Familial clustering of colon cancer is more common. The objective of this study is to determine the percent of high-risk colon cancer and the fraction of these high-risk cases with FAP, AFAP, and HNPCC from a unique genealogic-linked population based data set. Here, high-risk is defined as two first-degree relatives with colon cancer or one patient diagnosed with colon cancer at age 50 years or less. High-risk colon cancer patients were ascertained from Utah Population Database, which contains genealogy data, linked to the Utah Cancer Registry. To estimate the frequency of high-risk colon cancer, those cases meeting our high-risk criteria diagnosed 1966-1998 were divided by the total number of cases in that time frame resulting in a frequency of 25.5%. High-risk cases diagnosed 1965-1997 were invited to participate in research. Consent and pathology records were obtained on 648 cases, and tissue blocks or slides were collected on 385 cases. Review of medical records revealed two FAP cases (0.25%), one probable AFAP case, and 13 cases with several or >5 adenomatous polyps (2.0%). Pathology was confirmed and DNA extracted from normal and tumor regions of the tissue block. Microsatellite instability (MSI), a phenotype of HNPCC cancers, was determined on 270 of the cancers. MSI cancers constitute 13% (n=36) with an average diagnosis age of 58.5 yrs. The microsatellite stable cancers had an average diagnosis age of 59.5 yrs. We are currently using a combination of pedigree assessment, immunohistochemistry and sequencing of MLH1 and MSH2 in the MSI cases to estimate the frequency HNPCC. Six HNPCC cases from four kindreds (2.2%) have been confirmed and 5 are strongly suspected (4.1%). Since AFAP presents with a variable clinical phenotype, sequencing regions of the APC gene is currently underway to precisely estimate the fraction of AFAP in the high-risk population.

**BRCA1 and BRCA2 mutation and frequency profiles for the German population derived from more than 1000 case studies.** A. Meindl and representative of *The German Consortium for Hereditary Breast and Ovarian Cancer*. Abt Medizinische Genetik, Kinder Ludwig-Maximilians Univ, Munich, Germany Deutsche Krebshilfe, Bonn, Germany.

The Deutsche Krebshilfe supports a German-wide, multi-center study to establish a BRCA mutation profile and to determine family types with high frequencies of mutation in these genes. After screening more than 1000 unrelated index-patients from German breast/ovarian cancer families, deleterious mutations were found in about 320 cases. More than one third of these mutations may be specific for the German population. Twenty common mutations were found in 70% of the BRCA1 cases and 15 recurrent mutations in nearly 50% of the BRCA2 cases. Haplotype analysis strongly indicates that 15 out of 21 mutations originate from a common founder. Fifty-five rare variants with unknown relevance for tumorigenesis were found in another 80 families but not in 200 control individuals. As for most of them, no functional assays are available, these variants are currently characterized by LOH analysis of the corresponding tumor samples. A mutation frequency of 55% was identified in high risk families with both breast and ovarian cancer. The detection rate for high risk families with at least two premenopausal cases of breast cancer was about 40%. These data provide strong evidence for further predisposing genes in the German population and approaches have been implemented to identify such genes. These approaches include the determination of expression profiles in BRCA1/2 negative tumors and evaluation of candidate genes mapped to chromosome 8p and 11q. Less than 10% of mutations in the two known BRCA genes were found in families with two or three affected females, and having only a single or no premenopausal individuals. Families with three females affected by breast cancer can now be used for the detection of low penetrance genes by SNP analysis. In summary, the comprehensive analysis of more than a thousand families provides valuable data for genetic counselling and resources for the identification of novel genes associated with tumorigenesis.

**The Predictive Value of Established Clinical Criteria for BRCA1/2 analysis.** *S. Nanda*<sup>1</sup>, *P.J. Ainsworth*<sup>1, 2</sup>, *J.H. Jung*<sup>1</sup>, *N. Scanlan*<sup>3</sup>, *G. Sheridan*<sup>1</sup>. 1) Cancer Genetics, London Health Sciences Centre, London, ON, Canada; 2) Molecular Diagnostic Lab, London Health Sciences Centre, London, ON, Canada; 3) Cancer Genetics, London Regional Cancer Centre, London, ON, Canada.

The familial cancer histories and genotypic data for Southwestern Ontario kindreds, tested for mutations in BRCA1/2 genes between 05/96 and 05/01 were evaluated. We wished to determine if the clinical criteria established by the Ontario Cancer Genetics Network (OCGN), and more recently the Ontario Ministry of Health and Long Term Care (MOHLTC), adequately defined individuals/families at risk for the breast/ovarian syndrome. Hereditary Breast Cancer accounts for 5-10% of all cases of breast cancer and a large majority of these familial cases result from mutations in the BRCA1/2 genes. Statistical analysis was performed on 12 sub-groups outlined in the criteria. Of those eligible, a total of 401 individuals proceeded with BRCA gene testing. This was carried out by using a combination of the protein truncation test, utilizing both leukocyte genomic DNA and cDNA as template along with selective sequence analysis of genomic DNA. Individuals were recruited through the OCGN/MOHLTC and histopathological confirmations of a majority of cancers were made. 27 unique mutations in BRCA1 and 21 unique mutations in BRCA2 were detected. 119 of the 401 individuals tested demonstrated a mutation in one of the two BRCA genes, an overall positivity rate (PR) of 30%. The number of probands (individuals at highest risk who were tested first) was 225, had a 20% PR. In high risk groups significant findings include a 50% PR in families with two cases of ovarian cancer at any age in 1<sup>st</sup> or 2<sup>nd</sup> degree relatives. Individuals with both breast and ovarian cancer or bilateral breast cancer (the first breast tumour occurring at 50 or under) had a 42% PR. In an intermediate risk group, a 27% PR was observed in individuals with breast cancer, age 35 or under. A lower risk group, such as three breast cancers at any age in a pattern suggestive of inheritance, resulted in a 10% PR. These results indicate that our established clinical criteria are adequate in identifying at risk individuals in our population.

**Gynecological Malignancy as a "Sentinel Cancer" for Women with HNPCC.** *K. Lu<sup>1</sup>, M. Dinh<sup>1</sup>, W. Kohlmann<sup>1</sup>, P. Watson<sup>2</sup>, J. Green<sup>3</sup>, S. Syngal<sup>4</sup>, P. Bandipalliam<sup>4</sup>, L. Chen<sup>5</sup>, B. Allen<sup>5</sup>, P. Conrad<sup>5</sup>, J. Terdiman<sup>5</sup>, T. Burke<sup>1</sup>, D. Gershenson<sup>1</sup>, H. Lynch<sup>2</sup>, P. Lynch<sup>1</sup>, R. Broaddus<sup>1</sup>.* 1) MDACC, Houston, TX; 2) Creighton University, Omaha, NE; 3) University of Newfoundland, Canada; 4) Dana Farber Cancer Institute, Boston, MA; 5) UCSF, San Francisco, CA.

While HNPCC has been regarded as a colorectal cancer (CRC) dominated syndrome, endometrial cancer (EC) and ovarian cancer (OC) are also important components. Women with HNPCC have 40-60% and 12% lifetime risks for EC and OC, respectively. A significant number of women with HNPCC will have more than one cancer in their lifetime. Therefore, the occurrence of EC/OC as the first cancer in a young woman may indicate the presence of HNPCC. We hypothesize that EC and OC are "sentinel cancers" in women with HNPCC. The MDACC, Creighton, UCSF, Newfoundland, and Harvard registries were surveyed, and the results summarized below.

HNPCC families examined	223
Total # of women with CRC & EC/OC	126
CRC diagnosed first	47
EC/OC diagnosed first	57
CRC & EC/OC diagnosed simultaneously	22

These results show that in approximately 50% of the women studied, EC/OC was the "sentinel cancer", preceding the development of CRC. Therefore, gynecologists are essential in the identification of women with HNPCC. Such early identification is crucial for the optimal screening for subsequent cancers.



Program Nr: 395 from the 2001 ASHG Annual Meeting

**Hereditary Gastric Cancer. Results of molecular analysis and treatment.** *P. MacLeod.* Section of Genetics, Department of Laboratory Medicine, Victoria General Hospital, VICTORIA, BRITISH COLUMBIA., BRITISH COLUMBIA, Canada.

Hereditary Diffuse Gastric Cancer. Results of molecular analysis and treatment.

P.M. Macleod on behalf of the International Gastric Cancer Linkage Consortium. Division of Medical Genetics, Department of Laboratory Medicine, Victoria General Hospital. 1 Hospital Way, Victoria B.C. Canada V8R 6J4  
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The calcium dependent epithelial adhesion protein e-cadherin (ECAD) plays an important role as an invasion suppressor/tumor suppressor molecule.

Inherited mutations in the e-cadherin gene (CDH1) result in reduced expression of ECAD, and is regarded as one of the main molecular events involved in dysfunction of the cell-cell adhesion system triggering cancer invasion and metastasis. Germline mutations in CDH1 predispose to some forms of an autosomal dominant early onset diffuse gastric cancer syndrome (HDGC).

Family data were collected by member groups of the Consortium. Mutation analysis identified 21 truncating and 6 missense mutations. Preliminary data estimate the penetrance to be about of 70% and the mean age of diagnosis of metastatic cancer of 46 years. The risk of developing gastric cancer is 50% at age 36. Women carrying a mutation are also at significant risk for developing lobular breast cancer. Elective prophylactic gastrectomy solely on the basis of mutation analysis has been performed on 10 individuals. In each case, detailed histopathological examination demonstrated small foci of signet-ring cells indicative of premetastatic intramucosal carcinoma. These had been missed by previous endoscopic biopsies.

Based on this experience, we have developed guidelines for ascertainment of cases, genetic counseling and molecular testing and propose a strategy for clinical and surgical management of family members at risk for this syndrome. The Consortium is offering access to molecular testing and clinical followup for additional families.

**Is there an association between Hodgkin's disease and increased risk of general cancer? - Results from a Swedish population based case-control study.** A. Kingman<sup>1</sup>, K. Hemminki<sup>2</sup>, C. Dong<sup>2</sup>, Y. Shugart<sup>3</sup>. 1) NIDR, NIH, Bethesda, MD; 2) Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden; 3) Epidemiology Department, School of Public Health, Johns Hopkins University.

Genetic etiology of hematopoietic malignancies has been explored by various investigators (Haim et al 1982), (Eriksson and Hallberg 1992), (Lynch et al 1992), (Shpilberg et al 1994), (Mack et al 1995), (Shugart et al 2000). There have been a few attempts to look at familial occurrence of hematologic malignancies. The goal of this study is to determine whether general malignancies aggregate in families selected through probands affected with Hodgkin's lymphoma (HL) using the Family Cancer Database in Sweden (Hemminki and Vaitinen, 1998). A total of 1427 of HL diagnosed reported to the Swedish Cancer Register between 1940 and 1960 were collected, including 885 males and 542 females. Recorded information includes birth year, gender, county code, birth place, age of onset for first and second cancer, cancer types of both parents, age and birth place of each parent. For each case, a single control without any cancer was selected through a matching procedure by age and gender. Six hundred sixty three first-degree relatives affected with cancer among control families and 717 among case families were found. Analyses of covariances models were applied in the analysis of numbers of affected first-degree relatives in cases and control families. No association was detected between the HL status and the risk of general malignancies among parents, sibs or among all first-degree relatives of HL probands. However, after adjusting for differences in sibship size, the analysis suggested a weak association (P value =0.0971 ) between HL and first-degree relatives. -----  
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**Identification of a somatic mosaicism of the APC gene associated with adenomatous polyposis coli and germline transmission.** *S. Olschwang*<sup>1</sup>, *J. Bourguignon*<sup>2</sup>, *C. Boisson*<sup>1</sup>, *C. Martin*<sup>2</sup>, *T. Frebourg*<sup>2</sup>. 1) CEPH, Paris; 2) INSERM EMI 9906 IFRMP, Faculté de Médecine et de Pharmacie, Rouen, France.

De novo mutation of the APC gene is involved in approximately 25% of cases of adenomatous polyposis coli, post-zygotic events having been reported only twice. We report here a family in which the proband is bearing an APC post-zygotic mutation, present both in the germ-line and the colonic epithelium. This patient underwent total colectomy at age of 50 for diffuse adenomatous polyposis, that was revealed by a rectal adenocarcinoma. There was no previous family history of polyposis, suggesting that the corresponding mutation had occurred as a de novo event, but complete sequencing of the APC coding region on peripheral blood genomic DNA did not reveal any mutation. The subsequent follow-up of her 7 children found typical polyposis in 4 cases. At this stage, sequencing of the APC gene revealed a common deleterious mutation (Q541X, exon 12), present in all 4 affected children. DNA analysis of several peripheral blood samples from the proband confirmed the absence of the mutation, indicating the presence of a mosaicism with a germline component. Since the proband was herself affected, we postulated that the APC mutation should also be present within the colonic epithelium. Microdissection and direct sequencing of the APC gene confirmed the Q541X heterozygous mutation in the normal colonic epithelial cells and showed loss of the wild-type allele within the tumour cells. The mutation was absent of other colonic cells, such muscle. Thus, this mutation was only found in the germline and in the colonic epithelial cells. To our knowledge, this is the second report of a combined somatic/gonadal mosaicism for the APC gene (Farrington SM, and MG Dunlop. *Am. J. Hum. Genet.* 64:653-658, 1999), and the first report of a mosaicism affecting simultaneously the intestinal epithelium and the gonads, which respectively derived from the endoderm and the mesoderm. This observation is suggestive of an early cell migration abnormality, that could be documented only by the presence of a pathogenic mutation.

**APC mutations in Swedish FAP patients: Preliminary results.** *J. Meuller<sup>1</sup>, Y. Engwall<sup>1</sup>, A. Bergman<sup>1</sup>, J. Wahlström<sup>1</sup>, J. Björk<sup>2</sup>, M. Nordling<sup>1</sup>.* 1) Clinical Genetics, Goteborg University, Goteborg, Sweden; 2) Dep. Gastroenterology & Hepatology, Karolinska Hospital; Stockholm, Sweden.

Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited disease caused by germ line mutations in the APC gene. It predisposes to the development of hundreds to thousands of colorectal polyps, of which some will progress to cancer. Additionally, FAP-patients often develop extracolonic manifestation, such as desmoids, osteomas and retinal lesions. Since the nature and frequency of APC mutations in Swedish FAP patients are largely unknown a study was initiated in order to screen all families in the Swedish polyposis registry (approximately 250 families) for APC-mutations. Samples were initially screened for truncating mutations using the protein truncation test (PTT) followed by D-HPLC, SSCP or direct sequencing if needed. Detected mutations were verified by sequencing. To date 48 samples have been completely screened. Overall 67 % (32/48) of the patients were found to carry a disease causing APC mutation. Twenty-seven (27) different pathogenic mutations were detected, of which 16 has not been described previously. One patient with attenuated FAP was found to have a truncating mutation in exon 1 (nt70C>T) which to our knowledge is the first reported truncating mutation 5- to exon 3. In another case, low level mutational mosaicism (nt 2699-2700delCT) was demonstrated for a de-novo patient with attenuated FAP. The common mutations 1309 (3927-3931del AAAGA) and 1061(3183-3187delACAAA) were found in 6% (2/32) and 9% (3/32) cases respectively.

**BRCA1 and BRCA2 analysis in 523 Austrian HBOC and HBC families.** R. Kroiss<sup>1</sup>, T. Wagner<sup>1</sup>, D. Muhr<sup>1</sup>, E. Fleischmann<sup>1</sup>, V. Korn<sup>1</sup>, Austrian Hereditary Breast and Ovarian Cancer Group<sup>1</sup>, Th. Helbich<sup>3</sup>, Ch. Zielinski<sup>2</sup>, E. Kubista<sup>1</sup>, P. Oefner<sup>4</sup>. 1) Dep of OB & GYN, Division of Senology, and Ludwig Boltzmann Institute for Clinical Experimental Oncology, University of Vienna; VIENNA, Vienna, Austria; 2) Division of Oncology, Department of Medicine I; 3) Department of Radiology, University of Vienna; 4) Stanford DNA Sequencing and Technology Center, Palo Alto.

Using DHPLC the frequencies of BRCA1 and BRCA2 mutations in 268 Austrian families with hereditary breast cancer only (HBC) or breast and ovarian cancer (HBOC) were determined. Disease associated BRCA1 mutations were detected in 20% of all families. The percentage was highest in 60 HBOC families (40%) whereas the overall frequency of BRCA1 was lower in 193 HBC families (14%). In the BRCA2 gene apparently disease-associated mutations were identified in 9%. The percentage of disease-associated mutations was highest in families with male and female breast cancer (29%). In HBC families 7% could be explained by BRCA2 mutations. In HBOC families 10% BRCA2 mutations were detected. One BRCA2 founder mutation (8983-1CtoT) accounted for 36% of all BRCA2 families in Austria. Together 29% of all 268 families could be explained by BRCA1 and BRCA2 mutations. 523 HBC / HBOC families were screened for 9 recurrent BRCA1 mutations that account for 60% of all BRCA1 mutation families in Austria. In 59 (11%) families one of these 9 recurrent BRCA1 mutations could be identified. Cys61Gly in exon 5 could be detected in 13 families and 1806 CtoT in 11 families. Both mutations are found world wide. The third most frequent mutation is a frameshift mutation in exon 11 3135del4 (9 families, 15%) that so far was only detected in families from Austria. In BRCA1 and BRCA2 mutation families more than 160 carriers were identified. Two thirds of the female mutation carriers were already affected with cancer. BRCA1 mutation carriers had an earlier median age of onset for breast cancer with 38 years than BRCA2 mutation carriers with 45 years. The median age for healthy BRCA1 and BRCA2 carriers was similar, 38 years for BRCA1 and 39 years for BRCA2 carriers.

**New LFS criteria identify more p53 mutation positive families than the classical LFS criteria.** *S. Kiuru-Kuhlefelt, K. Aittomäki.* Department of Clinical Genetics, University of Helsinki and Helsinki University Central Hospital, Finland.

Li-Fraumeni Syndrome (LFS) is a dominantly inherited cancer predisposition syndrome with high penetrance. The classical criteria for LFS were proposed in 1988 by Li and Fraumeni. These included a proband with a sarcoma <45 years of age, a first degree relative with cancer before this age, and another first or second degree relative with either cancer <45 years of age or a sarcoma at any age. With such a highly penetrant cancer predisposition syndrome, affecting children and adolescents, false diagnosis should be avoided. Then again, it is important to identify individuals at risk of LFS to plan appropriate follow-up. As the molecular genetic diagnosis of LFS has become available, the classical criteria maybe too stringent and are likely to miss some LFS families. Therefore, there is a need for defining new criteria for genetic testing of LFS. Recently Chompret et al. proposed criteria for p53 mutation testing. These were: 1. A proband with a characteristic LFS-tumor (sarcoma, brain tumor, breast cancer, adrenocortical carcinoma) <36 years and at least one first or second degree relative with a characteristic LFS-tumor (other than breast cancer, if the proband had this) <46 years or with multiple tumors. 2. A proband with multiple tumors, two of which represent characteristic LFS-tumors and the first of which occurred before 36 years. 3. A proband with adrenocortical carcinoma whatever the age of onset or family history. Seven families with a suspicion of LFS were referred to our unit for genetic counselling and were tested for p53 mutations. While three of the families fulfilled the classical LFS criteria, all seven families fulfilled the criteria suggested by Chompret et al. A p53 mutation was found in five families. Only two fulfilled the classical criteria, whereas all five were identified by the new criteria. In two families, which also fulfilled the new criteria, no mutation could be detected. These results tentatively suggest that the criteria by Chompret et al. are useful in identifying families for p53 analysis, but need to be tested in a larger number of families.

**Detection of Deletions in hMLH1 and hMSH2 Genes by Multiplex Exon Probe Southern Hybridization.** *M. Mai, G. Chong, B. Bapat, KC. Halling, SN. Thibodeau.* Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First St. SW Rochester, MN. 55905.

Alterations in DNA repair genes directly contribute to the pathogenesis of hereditary nonpolyposis colorectal cancer (HNPCC). Alterations in hMLH1 and hMSH2 account for the majority of germline mutations detected in HNPCC, with large deletions occurring in a fraction of these patients. Although there are several methods available to detect hMLH1 and hMSH2 deletions, it is often difficult to detect the abnormality and to define the extent of the deletions on an exon by exon level. The goal of this study was to generate a limited number of probes for Southern blot analysis that would span the length of the hMLH1 and hMSH2 gene, and at the same time allow one to ascertain the extent of these deletions. To accomplish this, individual hMLH1 and hMSH2 exons were PCR-amplified, radioactively labeled, and hybridized to EcoR I, Bgl II or Hind III cut genomic DNA. Results revealed probe-enzyme combinations that would generate non-overlapping bands on Southern analysis. This information was used to create a recombinant DNA probe that contained equimolar amounts of 4 to 6 hMLH1 or hMSH2 exons and which would generate non-overlapping bands on Southern analysis. To create these probes, recombinant PCR was performed to link different exons together and the linked exons were then subcloned into pGEM-T easy and pCR 2.1-TOPO vectors. The recombinants and the exons they contain are as follows: MLH1 recombinant 1: exons 2, 4, 7, 11, 13, and 16, MLH1 recombinant 2: exons 1, 5, 9, 10, 15, and 18; MLH1 recombinant 3: exons 3, 6, 12, and 14; MSH2 recombinant 1: exons 2, 4, 10, 11, and 12, and 14; MSH2 recombinant 2: exons 1, 3, 5, 8, and 16; and MSH2 recombinant 3: exons 6, 7, 9, 13, and 15. We have tested these probes on patients with hMLH1 and hMSH2 alterations and found that they allow us to more clearly identify and define the extent of the deletion on an exon-by-exon basis.

**Cancer-associated somatic mutations of the NF1 gene.** *S. Han, D. Cooper, M. Upadhyaya.* Medical Genetics, Cardiff, Wales, UK.

NF1, an autosomal dominant disorder, affects 1 in 4000 individuals world-wide. The condition is characterised by multiple benign neurofibromas, café-au-lait spots, Lisch nodules and an increased risk of malignancy. The 350kb NF1 gene contains 60 exons and disease-causing mutations are spread across the gene. Neurofibromin, the NF1 gene product, appears to be involved in the control of growth and differentiation through down regulation of Ras. The NF1 germline mutation spectrum is now well characterised but only limited information is available on the nature of the somatic lesions. Somatic NF1 mutations have also been identified in several tumour types not usually associated with NF1 patients. How NF1 gene mutations contribute to the formation of both NF1-associated and un-related sporadic tumours is not known. Somatic mutational spectrum of the NF1 gene may aid in the identification of other functional domains of neurofibromin, may improve our understanding of the genotype/phenotype relationship in NF1 and would also allow us to ascertain whether the site of germline mutations determines the location and nature of somatic changes as has been reported in FAP. Denaturing high performance liquid chromatography (DHPLC) was used to screen the entire NF1 gene for mutations. The detection sensitivity of DHPLC was evaluated in a retrospective study of a cohort of 111 unrelated NF1 patients with known germline mutations; 97% of lesions were detected. In a subsequent prospective study, mutations were identified in 34 individuals (68%). We have screened 26 NF1 patients with multiple tumours and have identified somatic mutations in 30/67(45%) tumours. Loss of heterozygosity was identified in 21/67(37%) tumours using 8 intragenic polymorphic markers. Microlesions were identified in 9 tumours (R304X, 1541-2delAG, 1889delT, S637R, 2033delC, 4773-5C-T, Q1785X, R2429X), 5 of these lesions represent novel changes. Interestingly, the alteration 2033delC was identified in two tumours from the same patient and all the characterised alterations are located outside the GAP-related domain (encoded by exons 21-27a) of neurofibromin, the only domain with a known function. Our data suggest that there is a predominance of truncating somatic mutations in the NF1 gene.



**Difficult genetic counseling in a family with pathogenic BRCA2 mutation.** *I.I. Ingargiola<sup>1</sup>, M. Smaers<sup>1</sup>, P.J. Van Cangh<sup>2</sup>, C. Verellen-Dumoulin<sup>1</sup>, K. Dahan<sup>1</sup>.* 1) Dept Genetics, UCL Saint-Luc Hosp, Brussels, Belgium; 2) Dept Urology, UCL Saint-Luc Hosp, Brussels, Belgium.

The origin of a breast cancer is genetic in 5% of cases with an autosomal dominant inheritance. When a mutation is found in a family, the prevention is very important for the sibship and sibling. We have studied one family of 26 members. In this family 8 members have been affected by one or several cancers (4 breast cancers including one male, 2 lung cancers, 1 colorectal cancer, 1 endometrium cancer and 2 bladder cancers). The proband (K.E.) is a female who developed a primitive adenocarcinoma of endometrium when she was 49 years old. At age 66, she was affected by a primitive bladder cancer. Her sister (K.M.) was affected at age 66 by a primitive left breast cancer, at age 74 by a primitive left lung cancer and at age 75 by a primitive bladder cancer. The family history is highly suggestive of a hereditary cancer susceptibility syndrome. A search for mutation in the BRCA1, BRCA2 and P53 genes was carried out for K.E. We didn't find any mutation for the BRCA1 and P53 genes. By using PTT followed by a sequencing of exon 11 of BRCA2, an insertion of an adenine with apparition of a premature stop codon (TGA) in position 2003: 6171insA. This mutation was not observed in K.M. The difference between the two patients are: the age of apparition of cancers (the apparition is later for K.M. than for the proband K.E.) and the degree of differentiation of bladder carcinoma (more differentiated for the proband K.E.). **Conclusion** : Firstly we report a pathogenic BRCA2 mutation in a woman with 2 epithelial cancers not usually found in BRCA2 hereditary breast cancer. Secondly the sister with breast cancer isn't a carrier of the germline mutation after verification of new DNA samples. The 2 sisters have developed a transitional cell carcinoma of bladder after smoking exposure. These results should be explained by the existence in this family of another deleterious gene such as Mismatch Repair Gene either or by the coexistence of associated factors (genetic or environmental) responsible of a phenocopy.

***SDHC* functions as a tumor suppressor gene in autosomal-dominant paraganglioma, type 3.** *S. Niemann*<sup>1</sup>, *W. Kummer*<sup>2</sup>, *P. Rustin*<sup>3</sup>, *U. Müller*<sup>1</sup>. 1) University Giessen, Institute of Human Genetics, Giessen, Germany; 2) University Giessen, Institute of Anatomy and Cell Biology, Giessen, Germany; 3) INSERM U393 Hôpital des Enfants Malades, Paris, France.

Paragangliomas (PGLs) are usually benign, neural-crest-derived tumors of the paraganglionic system. Most common locations include the carotid bifurcation, the vagal nerve and the middle ear. PGLs occur both sporadically and in a hereditary, autosomal dominant pattern. There are at least 3 loci in autosomal dominant PGLs. Two loci are maternally imprinted and have been assigned to 11q23 (*PGL1*) and 11q13.1 (*PGL2*). *PGL1* is caused by mutations in *SDHD*, the small subunit of cytochrome b of mitochondrial complex II (succinate ubiquinone oxidoreductase). There is no evidence of imprinting at a third locus, *PGL3*. Applying a functional cloning approach we recently identified *SDHC* (encoding the large subunit of cytochrome b of mitochondrial complex II) as the disease gene in *PGL3*. A start codon mutation was discovered in constitutive DNA from all histologically verified patients. No transcript of the mutated allele was detected in both peripheral lymphocytes and tumor tissue. Furthermore, LoH was observed at several loci flanking *SDHC* in 1q21. In order to substantiate functional loss of both copies of *SDHC* in tumor tissue at the protein level, we performed immunofluorescence studies using anti-*SDHC* antiserum (rabbit anti-human *SDHC*). There was no immunoreactivity in parenchymal tumor cells. However, surrounding non-tumor cells were labelled. Furthermore, *SDHC* was clearly present in a total of 8 sporadic paragangliomas. The findings are consistent with *SDHC* functioning as a tumor suppressor in *PGL3*.

**Fanconi anemia D2 (*fancd2*) knockout mice have a phenotype more severe than *fanca* and *fancc* mice, but milder than *brca1* mutants.** *S. Houghtaling*<sup>1</sup>, *C. Timmers*<sup>1</sup>, *C. Reifsteck*<sup>1</sup>, *S. Olson*<sup>1</sup>, *S. Jones*<sup>2</sup>, *M. Grompe*<sup>1</sup>. 1) Molecular and Medical Genetics, OHSU, Portland, OR; 2) Transgenic-Knockout Animal Core Facility, UMass Medical School, Worcester, MA.

Fanconi anemia (FA) is an autosomal recessive chromosomal instability syndrome associated with birth defects, progressive bone marrow failure, and cancer susceptibility. Cells derived from FA patients show elevated levels of chromosomal breakage and an increased sensitivity to DNA cross-linking agents. Genes for 6 of 8 known complementation groups (*FANCA-G*) have been cloned. The recently cloned *FANCD2* gene consists of 44 exons and is alternatively spliced in both the human and mouse. After DNA damage, *FANCD2* is monoubiquitinated and then targeted to nuclear foci together with *BRCA1*. Wild-type function of the multimeric FA protein complex consisting of *FANCA*, *C*, *E*, *F* and *G* is required for this *FANCD2* modification. To understand the *in vivo* role of this gene, we have disrupted *fancd2* by homologous recombination and generated mice homozygous for the targeted allele. In contrast to *brca1* knockouts, viable *fancd2* mutants were born in expected Mendelian ratios. The phenotype of *fancd2* mutant mice was similar to *fanca* and *fancc* mutant mice but was more severe. Gonads of both sexes showed decreased numbers of germ cells, but no other morphological abnormalities were found. The testicular volume and histology of testes was significantly different from either *fanca* or *fancc* mutants. Testicular weights were 97 +/- 4 mg in controls, 80 +/- 3 mg in *fanca*, 56 +/- 4 mg in *fancc* and 16 +/- 2 mg in *fancd2* mutants. Fibroblasts from *fancd2* mutant mice were also more sensitive to DNA crosslinkers. Primary cells were clearly sensitive to O<sub>2</sub> in culture. No obvious hematological abnormalities nor tumor development have been observed up to 3 months of age. The phenotype of the *fancd2* mutant mice supports the presumed role of the FA proteins in response to DNA damage. Together, the genetic and biochemical data suggest that wild-type *BRCA1* is required for function of the FA pathway, possibly as an ubiquitin ligase. In contrast, neither *FANCC*, *FANCA* or *FANCD2* are necessary for *BRCA1* function.

**Novel pathogenic missense mutations in BRCA1 and BRCA2 perturb three different aspects of protein function.**

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Most BRCA1 and BRCA2 germline mutations detected by conventional sequencing are nonsense mutations or small insertions or deletions that lead to frameshifts. A few missense mutations that predispose to breast and ovarian cancer have been observed in high risk families, particularly substitutions of the cysteine residues of site II of the RING finger domain. Among our families at high risk of breast and ovarian cancer, we have recently identified three novel missense mutations of BRCA1 and BRCA2 that perturb three different aspects of protein function. BRCA1 Leu1407Pro was inherited by four women who developed ovarian cancer (dx ages 42-76) in an exclusively ovarian cancer kindred of English ancestry (Family 350). The leucine at codon 1407 is evolutionarily conserved and lies in the middle of a coiled-coil domain critical to transcriptional activation function of BRCA1 (Hu et al., JBC 275:40910,2000). Substitution of proline for leucine disrupts the coiled-coil structure and severely impaired the activation function. Insofar as we know, Leu1407Pro is private in family 350. It was not present in 75 ovarian tumors, 65 breast cancer probands with a family history of ovarian cancer, and 400 breast cancer cases that includes Caucasian and African American women. BRCA1 Cys44Phe was identified in a family of Arab ancestry (Israeli family 4) with four cases of breast cancer (dx ages 30-35) and three cases of ovarian cancer (dx ages 22-40). Cys44 is a conserved residue of site I of the BRCA1 RING domain. Mutations in site I are residues are predicted to have more severe effects on RING domain structure than do mutations in site II. We suggest this mutation be tested in breast or ovarian cancer families of Arab ancestry. BRCA2 Met1Arg was inherited by five women with breast cancer (dx ages 41-55) in a family of Mexican ancestry (Family 6). Because this mutation destroys the wildtype start codon, translation is predicted to begin at codon 124. If the mutant protein is translated it would lack the putative transactivation domain encoded by exon 3.

**Phenotypic variation between two probands with FAP due to large deletions in exon 15 of the APC gene.** W.K. Kohlmann, P.A. Ward, P.M. Lynch, L-K. Su. University of Texas MD Anderson Cancer Ctr, Houston, TX.

Familial adenomatous polyposis (FAP) is caused by germline mutations at *APC*. FAP patients typically present with 100s to 1000s of colorectal polyps at a young age. However, patients carrying germline *APC* mutations at the first 4 exons, the alternatively spliced region of exon 9 and the 3' half of exon 15 usually develop an attenuated phenotype. We report two female probands with large deletions of exon 15 in *APC* who have highly divergent phenotypes. Proband 1 presented at age 21 with rectal bleeding, at which time colonoscopy identified innumerable polyps in her rectum and multiple small adenomas throughout the remaining colon. At age 55, Proband 1 was found to have severe villous adenomas of the duodenum and ampulla. The patient also has a history of epidermal cysts. Based on the family history, her disease appears to be due to a new mutation. She has had one affected son who has a similar expression of the disease. Proband 2 presented at age 42 with rectal bleeding, and colonoscopy detected between 70-90 polyps predominantly in the right colon with sparing of the rectum. Pathology of one polyp indicated adenocarcinoma. Proband 2 had a normal stomach and duodenum at time of diagnosis. She has a significant family history of colon cancer, with the average age of diagnosis 46 years, ranging from 36 to 59. Polyp number in the family also varies from the attenuated presentation of the proband to approximately 300 polyps in her brother at age 38. Mutation analysis of the entire *APC* coding region using protein truncating test did not identify a mutation in either family. By analyzing cDNA and genomic DNA for intragenic polymorphic nucleotides and genome walking, we found that these two families carried two different large deletions at *APC* that both deleted the entire exon 15. The variations between these two probands and within the family of Proband 2 suggests that other genetic factors may interact with the *APC* gene to determine the phenotype of an individual.

**Sublocalization of *LOH18CR*, a tumor suppressor gene implicated in osteosarcoma, to a minimal region between *D18S87* and *D18S42*.** M.J. Nellissery<sup>1</sup>, T.L. Johnson-Pais<sup>2</sup>, C. Buller<sup>2</sup>, P. Bhatia<sup>1</sup>, D.G. Ammerman<sup>1</sup>, A.M.

Deshpande<sup>1</sup>, D. Pathmanathan<sup>1</sup>, R.S. Benjamin<sup>3</sup>, R. Gorlick<sup>4</sup>, P.A. Myers<sup>4</sup>, R.J. Leach<sup>2</sup>, M.F. Hansen<sup>1</sup>. 1) Center for Molecular Medicine, Univ. CT Health Center, Farmington, CT; 2) Department of Cellular and Structural Biology, Univ. TX Health Science Center, San Antonio, TX; 3) Department of Sarcoma Medical Oncology, Univ. TX M.D. Anderson Cancer Center, Houston, TX; 4) Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY.

Previous analysis of tumor-specific loss of constitutional heterozygosity (LOH) had identified a putative tumor suppressor gene (*LOH18CR*) that mapped to a subregion of chromosome 18q21.3-q22.1 that was linked to both familial Paget's Disease of Bone and Familial Expansile Osteolysis. We have developed a minimal tiling BAC contig which spans this critical region of LOH. Eleven new highly polymorphic STR loci were identified within this minimal region of LOH using sequence data from our BAC contig and screening the contig by BLAST analysis. These new polymorphisms have enabled us to refine the minimal region of LOH which must contain *LOH18CR* to a chromosomal region distal to *D18S87* and proximal to *D18S42*. This region appears to exclude *TNFRSF11A* (*RANK*) and *BCL2*, but includes *SKD1*, *serpin B5* (*maspin*), *serpin B13* and *serpin B3*.

***BRCA1* and *BRCA2* founder mutations are not associated with a high risk of colorectal cancer.** *B.L. Niell<sup>1</sup>, G. Rennert<sup>2</sup>, L.P. Tomsho<sup>1</sup>, J.D. Bonner<sup>1</sup>, S.B. Gruber<sup>1</sup>*. 1) University of Michigan, Ann Arbor, MI; 2) Department of Community Medicine and Epidemiology, Carmel Medical Center and Technion Faculty of Medicine, Haifa, Israel.

Mutations in *BRCA1* and *BRCA2* profoundly increase the risks of breast and ovarian cancers among women, but it is not clear whether mutations in these genes increase the risk of colorectal cancer (CRC). Three founder mutations, *BRCA1* 185delAG and 5382insC, and *BRCA2* 6174delT, have a combined prevalence exceeding 2% among Ashkenazi Jews, permitting analysis of cancer-specific odds ratios in case-control studies. The relationship of these three founder mutations to CRC was examined in the Molecular Epidemiology of Colorectal Cancer (MECC) study, a population-based case-control study in northern Israel. Eligible cases from this ongoing study included 529 Ashkenazi Jews diagnosed with incident CRC in northern Israel between March 1998 and March 2001. Controls were 386 age, sex and clinic-matched Ashkenazi individuals without CRC. Genotyping for 185delAG, 6174delT, and 5382insC was performed by PCR restriction analysis, allele specific PCR, and allele specific oligonucleotide hybridization, respectively. Genotyping was successful for 513 (97%) cases and 377 (98%) controls. No significant associations between *BRCA1* or *BRCA2* founder mutations and CRC were identified. Twelve (2.3%) cases and ten (2.7%) controls carried one of the three mutations, yielding an odds ratio = 0.9 (95% confidence interval 0.4 - 2.1). Analyses stratified by specific mutation had limited power, especially for 5382insC, but no strong relationships with CRC were identified for 185delAG, 5382insC, or 6174delT. Adjustment for age did not measurably change the results of this unmatched, interim analysis. In conclusion, these data provide direct evidence that these three *BRCA* founder mutations do not confer a risk as high as the 4-fold increase that has been reported elsewhere in the literature. Furthermore, it is unlikely that carriers of these founder mutations in *BRCA1* or *BRCA2* are at an increased risk of colorectal cancer.

**Genome-wide screen with tetra-nucleotide repeats demonstrates infrequent allelic imbalance in benign peripheral nerve sheath tumors in NF1.** *M.A. LIEW<sup>1</sup>, K. TANITO<sup>1,2</sup>, Y. ZHANG<sup>1</sup>, M.N. HANG<sup>1</sup>, L. BALLARD<sup>1</sup>, S. SAWADA<sup>2</sup>, M. NIIMURA<sup>2</sup>, D. VISKOCHIL<sup>1</sup>.* 1) PEDIATRICS, UNIVERSITY OF UTAH, SALT LAKE CITY, UT; 2) DERMATOLOGY, JIKEI UNIVERSITY, TOKYO, JAPAN.

Approximately 20-25% of patients with neurofibromatosis type 1 (NF1) have benign peripheral nerve sheath tumors (PNSTs), also known as plexiform neurofibromas. Some of these tumors undergo transformation into malignant PNSTs (MPNSTs), however pathogenesis of malignant transformation has not been determined. Stable benign PNSTs have double inactivation of NF1, therefore we hypothesize that additional genetic changes must occur to lead to malignant transformation. A genome-wide tetra-nucleotide genotyping screen was implemented to evaluate allelic imbalance in DNA derived from different sites within 8 benign PNSTs from 8 individuals with NF1. Using Genotyper<sup>TM</sup> software the area under the allele-specific peaks and the ratio of the areas for two alleles was compared between blood and tumor DNA to estimate allelic imbalance in tumor-derived DNA template. A ratio of peak areas for informative alleles that was either less than 0.75 or greater than 1.25 in tumor versus blood DNA samples was scored as allelic imbalance. Loss of heterozygosity (LOH) was scored by a ratio less than 0.2, or greater than 5.0. This allele imbalance could reflect either LOH or extensive amplification of one allele.

We genotyped 39 tetra-nucleotide markers from the distal arms of 22 chromosomes. Chromosomes 2q and 20p were not represented in the screen. 2/8 PNSTs had LOH, 1 PNST had one marker with LOH and 3 markers with allelic imbalance, while the other had 9 markers with LOH and 12 markers with allelic imbalance. The remaining PNSTs had 1-8 sites of allelic imbalance. These data were unable to identify common markers in PNSTs, but may be used to potentially classify the malignant state of a tumor based on the number of markers that show a change. Use of these markers with adjacent sets of markers in an extended set of tumors would better define candidate genes that might contribute to NF1-related tumor formation and growth.



**Is the Fanconi anemia DNA damage response pathway independent of p53 regulation?** *S. Komaki, Y.M. Akkari, R.L. Bateman, M. Grompe.* Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR.

Fanconi anemia (FA) is an autosomal recessive disorder with a complex phenotype including bone marrow failure and cancer proneness. FA is genetically heterogeneous, and to date at least 8 complementation groups (FANCA-C, D1, D2 and E-G) have been identified. FA cells are hypersensitive to agents which generate interstrand DNA cross-links (ICL) such as mitomycin-C (MMC) and psoralen. The p53 protein has a multiple important functions in genomic instability including G1/S, G2/M and mitotic checkpoints after DNA damage. MMC treatment has been reported to induce increased p53 mRNA levels in different cell types. This raises the question, whether p53 is important in cellular responses to ICL. Several groups have published experiments addressing the question of a possible interaction between the FA and p53 pathways, but no consistent interactions were found. To further address this issue we have cross-bred p53 deficient and FAC mutant mice. FANCC/p53 double mutant mice showed no embryonic lethality and no obvious phenotypic differences between p53 mutants and FANCC/p53 double mutants at birth. The survival of mice lacking of p53 protein but either mutant, heterozygous or wild type mice in FANCC was compared. The average survival of double mutants was 128 days as compared to p53 mutants 159 days ( $p < 0.05$ ), indicating modestly increased genomic instability in double mutants. However, the survival of p53 heterozygotes, did not differ significantly depending on the FANCC genotype. Cells from different combinations of FANCC and p53 genotypes were examined for their response to ionizing radiation (IR) or ICL. Wild-type and FANCC mutant cells but not p53 mutant cells displayed a long-lasting 4N cell cycle arrest after ICL treatment. Interestingly, FANCC/p53 double mutant cells also arrested after IR or ICL treatment. This suggests that FANCC works independently of p53 during growth arrest after both IR and ICL treatment. Together these data suggest that p53 function is at least partially dispensable in FANCC mutant cells and that FANCC allele provides a p53 independent mechanisms of cell cycle arrest after ICL or IR treatment.

**Database to study genetic susceptibility to Gastric Cancer.** *M. Jaju*<sup>1</sup>, *A.R. Sepulveda*<sup>2</sup>, *D.Y. Graham*<sup>3</sup>, *L.E. Peterson*<sup>1</sup>. 1) Chronic Disease Prevention and Control Research Center, Dept of Medicine, Baylor College of Medicine, Houston, TX; 2) Dept of Pathology, University of Pittsburg Medical Center, Pittsburg, PA; 3) VA Medical Center, Houston, TX.

Gastric cancer remains one of the human tumors with worst prognosis having 5-year survival rates typically below 20 percent. It is not clear if gastric cancer aggregates in families due to genetic inheritance, shared common environmental factors, or both. Several studies indicate involvement of genes and interactive factors like H.pylori. The first step towards the search for susceptibility genes is to identify families with a positive history of the cancer. In this study, patients diagnosed and treated for gastric cancer at the Methodist hospital, Houston, TX since 1990 were selected from the cancer registry. Patients for this study were recruited after obtaining IRB approval and informed consent. A detailed cancer family history questionnaire was completed and family history was confirmed by telephone interview. The second step of this study was to design a relational database using Microsoft Access software. The data obtained was organized in the form and report to analyze using database queries. The response rate from the 200 patients invited was 12 percent. These 9 families have strong family histories of gastric cancer as well as other cancers such as breast, prostate, leukemia and lymphoma. The data will be employed for segregation analysis and blood samples for single nucleotide polymorphisms and other genetic linkage studies. The detail of the preparation of the relational database and the significant factors that influence the data analysis will be shown.

**A new syndrome associated with autosomal dominant inheritance of familial paraganglioma and gastric stromal sarcoma.** *C.A. Stratakis<sup>1</sup>, J.A. Carney<sup>2</sup>.* 1) UGEN/DEB, NICHD, NIH, Bethesda, MD; 2) Mayo Clinic, Rochester, MN.

Paragangliomas occur in von Hippel-Lindau disease, neurofibromatosis and in 3 other syndromes, PGL-1, -2, and -3, all familial conditions. This report describes 12 patients, 7 men and 5 women, aged 10 to 46 years, from 5 unrelated families that manifested paraganglioma and gastric stromal sarcoma. Three sibships and 2 consecutive generations in each of 2 families were affected. Seven patients had paraganglioma, four had paraganglioma and gastric stromal sarcoma (1 from each of 4 families), and 1 patient had gastric stromal sarcoma. The 4 patients with both tumors were each misdiagnosed as having the Carney triad. The paragangliomas were transmitted in a manner consistent with autosomal dominant inheritance and incomplete penetrance. The paragangliomas were the presenting tumor in 9 patients (a symptomless mass in 6 and hypertension in 3). Eight patients had multiple tumors, located in the neck (18), retroperitoneum (8), mediastinum (2), and adrenal (1). The tumor functioned in 7 patients. Two paragangliomas "recurred" following surgery. None metastasized. The gastric tumors occurred on the lesser curvature and in the antrum, caused mucosal ulceration, bleeding and anemia in 4 patients, were multiple in 4, recurred twice in 1 patient, and metastasized to local lymph nodes and peritoneum in 3. Eleven patients (92 %) are alive, 4 with, or probably with, untreated or residual paraganglioma and 1 with recurrent gastric stromal sarcoma and paraganglioma. One patient succumbed to recurrent paraganglioma and postoperative neurologic sequelae. Because of the rarity of gastric stromal sarcoma, its multifocality, and the unlikelihood of coincidental co-occurrence of paraganglioma and gastric stromal sarcoma, it is very probable that the 2 neoplasms constitute a novel familial syndrome distinct from the familial PGLs or the previously described Carney triad.

**Identification of a deletion in the DNA mismatch repair gene MSH2 by Southern blot analysis.** R.E. Pyatt<sup>1</sup>, H. Hampel<sup>2</sup>, M. Sedra<sup>1</sup>, R.W. Schafer<sup>1</sup>, M.B. Fuchik<sup>1</sup>, I. Comeras<sup>2</sup>, A. de la Chapelle<sup>2</sup>, T.W. Prior<sup>1</sup>. 1) Department of Pathology, Ohio State Univ, Columbus, OH; 2) Division of Human Cancer Genetics, Ohio State Univ, Columbus, OH.

Hereditary nonpolyposis colorectal cancer (HNPCC) is characterized by tumors demonstrating microsatellite instability (MSI) and inherited mutations in one of the seven DNA mismatch repair genes: primarily MLH1 or MSH2. While extensive research has been done identifying small types of mutations in these genes, there is little information in the literature examining the role of large, genomic rearrangements in the etiology of HNPCC. In this study, we report the identification of a large, genomic deletion encompassing the first two exons of MSH2 in multiple members of an Ohio family. Lymphocyte DNA from an individual with an MSI + mucinous colon adenocarcinoma was examined by Southern blot analysis using a cDNA probe spanning the first seven exons of MSH2. On a *HindIII* digest, compared to the pattern seen in 110 normal controls, a new, unique band was observed and subsequently shown to be maternally inherited from an obligate carrier diagnosed with a transitional cell carcinoma of the ureter. Both smaller cDNA probes and exon specific ones were used to demonstrate that probes containing exon 3 would specifically hybridize to the unique band, while probes to either exon 2 or exon 4 would not. Additional unique bands were identified on *NsiI* and *AflIII* digests and these results suggested the presence of a deletion involving MSH2 exons 1 and 2 in this family. Quantitative PCR reactions to exon 2 demonstrated a reduction in product by approximately one half compared to normal controls while similar reactions to exon 3 showed no such reduction. While the deletion breakpoints were being identified by a vectorette PCR strategy, 5 siblings of the original individual requested the analysis of their carrier status. Southern blots were performed using *HindIII* digests hybridized with the full-length MSH2 probe and identified the unique band in 1 of 5 additional siblings. This study further supports the role of large, genomic rearrangements in the DNA mismatch repair genes as a cause of HNPCC.

**Evaluation of breast cancer linkage to the putative BRCA3 locus on chromosome 13q21 in 128 multiple case families from the International BRCA3 Linkage Consortium.** *C.I. Szabo<sup>1</sup>, D. Thompson<sup>2</sup>, J. Mangion<sup>3</sup>, R. Oldenberg<sup>4</sup>, F. Odefrey<sup>1</sup>, S. Seal<sup>3</sup>, R. Barfoot<sup>3</sup>, D. Teare<sup>2</sup>, B. Weber<sup>5</sup>, P. Devilee<sup>4</sup>, D. Easton<sup>2</sup>, D. Goldgar<sup>1</sup>, M. Stratton<sup>3</sup> and International BRCA3 Linkage Consortium (IBXLC).* 1) International Agency for Research on Cancer, Lyon, France; 2) Strangeways Reserch Laboratories, University of Cambridge, UK; 3) Institute of Cancer Research, Sutton, UK; 4) Leiden University, Leiden, Netherlands; 5) Abramson Family Cancer Research Institute, University of Pennsylvania, PA.

The known susceptibility genes for breast cancer, including BRCA1 and BRCA2, only account for a minority of the familial aggregation of the disease. Hence, other genes remain to be identified. The recent report of a putative BRCA3 locus on chromosome 13q21 (Kainu et al., 2000) that may account for 65% of non-BRCA1/BRCA2 families has raised expectations that a major breast cancer susceptibility gene has been localized. We have evaluated the contribution of this candidate BRCA3 locus to breast cancer susceptibility in 128 high-risk breast cancer families of Western European ancestry with no detected BRCA1 or BRCA2 mutation. These families consist of 650 women affected with breast cancer (median = 5/family), with 56% diagnosed under age 50. Samples from 409 affected individuals were analyzed for linkage to 13q21, yielding an overall multipoint LOD score of  $-38.0$  at D13S1308. No evidence of linkage under locus heterogeneity was found (estimated proportion of linked families,  $a=0$ ; upper 95% confidence limit of 0.13). Adjustment for possible bias due to selection of families on the basis of linkage evidence at BRCA2 did not materially alter this result ( $a=0$ , upper 95% CL 0.17). The previously reported  $a$  of 0.65 is clearly excluded in our study (HLOD =  $-11.0$ , corrected HLOD =  $-7.6$ ). In summary, we find no evidence of a breast cancer susceptibility locus on 13q21. However, if such a gene does exist, it is likely to account for only a small proportion of all non-BRCA1/2 families, indicating that other susceptibility loci must exist. Further linkage studies in large series of multiple case families will be needed to identify remaining genes underlying familial aggregation of the disease.

**Functional significance of a novel H718Y hMLH1 alteration associated with increased colorectal cancer risk among African-Americans.** *T.K. Weber<sup>1</sup>, Z. Yuan<sup>1</sup>, D. McDermott<sup>1</sup>, N. Petrelli<sup>2</sup>, M. Rodriguez-Bigas<sup>2</sup>, B. Keitz<sup>2</sup>, T.S. Ravikumar<sup>1</sup>.* 1) Department of Surgery and Molecular Genetics, Ullmann 707, Albert Einstein College of Medicine, New York, NY; 2) Department of Surgical Oncology, Roswell Park Cancer Institute, State University of New York at Buffalo, Buffalo, NY.

**Introduction:** Germline mutations in the mismatch repair(MMR) genes, hMLH1 and hMSH2 are associated with significant increased risk of colorectal cancer. The American Cancer Society reports African-Americans sustain the highest rates of colorectal cancer incidence and mortality of any U.S. population group. We previously reported (JAMA 1999 281:2316-2320) a novel highly conserved hMLH1 H718Y alteration detected in 3 unrelated African- American early age of onset colorectal cancer patients. In our present study we utilized a *Saccharomyces cerevisiae* dominant mutator model to assess the functional significance of the H718Y alteration. **Methods and Results:** hMLH1 cDNA derived from a patient with a heterozygous hMLH1 718 alteration, CAC->TAC or from a normal donor without a hMLH1 alteration was expressed in a MMR proficient strain with the reporter plasmids. The resultant transformants were assayed on a plate containing X-gal. Transformants expressing the wild type hMLH1 uniformly appeared blue. Transformants carrying the H718Y alteration produced white colonies, indicating compromised or absent hMLH1 function. **Conclusion:** This results suggests the hMLH1 H718Y alteration is associated with compromised MMR function. To date the H718Y alteration has only been reported in African-American probands. This suggests a specific population association reminiscent of the I1307K APC gene colorectal cancer association reported among Ashkenazi Jews. Further investigations including additional yeast based functional assays, case control association studies and clarification of true population frequency of H718Y are underway in our laboratory. Confirmation of loss of function would allow the H718Y alteration to serve in the identification of individuals at increased risk of developing colorectal cancer and facilitate effective clinical surveillance for those most in need.

**Improved hMLH1 and hMSH2 mutation detection using denaturing high performance liquid chromatography (DHPLC) analysis.** Z. Yuan<sup>1</sup>, TS. Ravikumar<sup>1</sup>, D. McDermott<sup>2</sup>, N. Petrelli<sup>2</sup>, M. Rodriguez-Bigas<sup>2</sup>, H.M. Chin<sup>2</sup>, L. Hazard<sup>2</sup>, B. Keitz<sup>2</sup>, L. Cuomo<sup>2</sup>, T.K. Weber<sup>1</sup>. 1) Department of Molecular Genetics & Surgery, Ullmann 707, Albert Einstein College of Medicine, New York, NY; 2) Department of Surgical Oncology, Roswell Park Cancer Institute, State University of New York at Buffalo, Buffalo, NY.

**Introduction:** Supply, labor and time costs present significant challenges to efficient and reliable laboratory germline mutation detection. Denaturing high performance liquid chromatography (DHPLC) is a new high-throughput technique for gene mutation scanning. In this study we report a novel analysis of DHPLC as a mutation detection method comparing it directly to single strand conformation polymorphism (SSCP) analysis. **Methods:** All 35 exons of the hMLH1 and hMSH2 genes respectively were amplified in 31 early age of onset colorectal cancer patients. Each of the 1085 amplified exons were screened twice, initially using SSCP and independently a second time using DHPLC analysis. All SSCP and DHPLC derived candidate sequence variations were subjected to direct nucleotide sequencing for confirmation. **Results:** SSCP suggested alterations in 168 of the 1085 exons studied (15%). However, subsequent nucleotide sequencing confirmed sequence changes in only 28 of these 168 exons for a false positive rate of 82%. DHPLC identified each of the 28 sequence confirmed alterations. DHPLC analysis of the entire 1085 exons compliment produced no false positive results. However, an additional 5 unique exon alterations (not detected by SSCP) were identified by DHPLC analysis and confirmed by direct sequencing. **Conclusion:** These results indicate a clear specificity advantage for DHPLC over SSCP based exon mutation screening of the MMR genes hMSH2 & hMLH1. The very high rate of SSCP based false positives translates into considerable costs that are avoided when DHPLC is utilized as no false positives were detected with its use. In addition, our results suggest DHPLC also provides increased sensitivity over SSCP. We conclude DHPLC offers multiple advantages compared to SSCP and deserves further study as a method of first choice for MMR gene exon mutation screening.

**Genetic analysis of BRCA1 and BRCA2 in 100 males with breast cancer.** *C.R. Richardson, A.M. Deffenbaugh, T.S. Frank.* Myriad Genetic Laboratories, Inc., Salt Lake City, UT.

Numerous studies have associated male breast cancer with the presence of germline mutations in BRCA2 but the contribution of mutations in the BRCA1 gene remains unclear. The aims of this study are to determine the contribution of BRCA1 and BRCA2 mutations in males with breast cancer, and to correlate mutation status with the reported family history information. A total of 100 males with breast cancer who underwent genetic testing between June 1997 to May 2001 were studied. The males were tested through full sequence analysis of the coding and adjacent non-coding regions of both BRCA1 and BRCA2 (n=70), sequence analysis of either BRCA1 or BRCA2 only (n=11), or analysis of three founder mutations prevalent in the Ashkenazi Jewish population (n=19). Clinical information was obtained through a test requisition form submitted with each sample. Overall, 25 males harbored deleterious BRCA mutations, consisting of 8 with mutations in BRCA1 only, 15 with mutations in BRCA2 only, and 2 with one mutation in each. The median ages of diagnosis were 51 years for men with mutations in BRCA1, 58 years for men with mutations in BRCA2, and 59 years for men in whom no mutations were identified. Of the 31 males reporting Ashkenazi Jewish ancestry, 11 (35.5%) were positive for BRCA1 or BRCA2 mutations, with one patient harboring a mutation in both BRCA1 and BRCA2. Of the 12 mutations seen in this group, 11 were one of the three founder mutations (187delAG, 5385insC, or 6174delT). Of the 69 males who did not specify Ashkenazi Jewish ancestry, 14 (20.3%) were positive for BRCA1 or BRCA2 mutations, one of whom carried one mutation in each gene. Mutations were reported in 2 Ashkenazi Jewish males with breast cancer who indicated a negative family history of breast or ovarian cancer. In contrast, no mutations were identified in the non-Ashkenazi males who reported a negative family history. Mutations in BRCA1 as well as BRCA2 are responsible for a significant proportion of men with breast cancer tested in a clinical setting. Mutations in BRCA1 and BRCA2 are overall more prevalent in men of Ashkenazi ancestry, and are especially associated with a family history of breast or ovarian cancer.



**Protocols for the investigation of large, genomic rearrangements in the DNA mismatch repair gene MLH3 by Southern blot analysis.** *T.W. Prior, M. Sedra, M.B. Fuchik, R.E. Pyatt.* Department of Pathology, The Ohio State University, Columbus, Ohio.

Hereditary nonpolyposis colorectal cancer (HNPCC) is characterized by an early age of onset and tumors demonstrating microsatellite instability (MSI). While tumors in HNPCC are primarily caused by inherited mutations in three of the six DNA mismatch repair genes (MLH1, MSH2, and MSH6), a new DNA mismatch gene termed MLH3 was recently identified and its role in HNPCC remains unknown. In this study, we report the development of Southern blot protocols for the analysis of large, genomic alterations in MLH3. Two cDNA probes were designed to encompass a majority of the gene with the first spanning exon 1 (1.8 Kb) and the second extending from the 3' portion of exon 1 to exon 10 (2.2 Kb). Using these probes, the hybridization patterns for 20 normal controls were defined on either *Hind III*, *NsiI*, *BclI*, or *PvuII* restriction enzyme digests with limited polymorphic variation noted. Smaller MLH3 cDNA probes, exon specific probes, and sequence information from both Human genome projects were used to identify the exact exons corresponding to each band seen on these patterns. Individuals manifesting MSI+ colorectal cancer who were negative for MLH1 and MSH2 mutations both by direct genomic sequencing and Southern blot analysis are currently being analyzed for the presence of genomic alterations in MLH3. The protocols outlined here demonstrate effective means by which to address the involvement of large, genomic alterations in MLH3 in the etiology of HNPCC.

**Allele Class-independent Intrafamilial correlation of Age at Onset, Age at Hearing Loss and Number of Intracranial Meningiomas in neurofibromatosis 2 (NF2).** *Y. Zhao*<sup>1</sup>, *R.A. Kumar*<sup>2</sup>, *M.E. Baser*<sup>3</sup>, *D.G.R. Evans*<sup>4</sup>, *A. Wallace*<sup>4</sup>, *G.A. Rouleau*<sup>5</sup>, *V.F. Mautner*<sup>6</sup>, *L. Kluwe*<sup>7</sup>, *H. Joe*<sup>1</sup>, *J.M. Friedman*<sup>2</sup>. 1) Dept of Statistics, U. of British Columbia, Vancouver, BC, Canada; 2) Dept of Medical Genetics, U. of British Columbia, Vancouver, BC, Canada; 3) Los Angeles, California; 4) Dept of Medical Genetics, St. Mary's Hosp, Manchester, UK; 5) Ctr. for Research in Neuroscience, McGill U., Quebec, Canada; 6) Dept. of Neurology, Klinikum Nord Ochsenszoll, Hamburg, Germany; 7) Dept. of Neurosurgery, University Hosp. Eppendorf, Hamburg, Germany.

Mutation of the *NF2* tumour suppressor gene cause NF2, an autosomal dominant disease characterized by very high frequencies of vestibular schwannomas, schwannomas of other nerves, meningiomas, and gliomas. The disease is highly penetrant but manifests variable expressivity both within and between families. We have developed statistical models to quantify familial correlations of age at onset of the presenting symptom, age at onset of hearing loss, and number of intracranial meningiomas. A random effects model employing survival techniques to account for right censoring was used for age at onset of the presenting symptom and age at hearing loss. A negative-binomial gamma mixture model with penetrance dependent on time since onset was used for number of meningiomas.

Using these models, we demonstrated a significant intrafamilial correlation for each of the three features in 377 NF2 non-probands from 154 unrelated families: age at onset ( $\tau = 0.36$ ; 95% confidence interval 0.23-0.47), age at hearing loss (0.40; 0.23-0.55) and number of meningiomas (0.20; 0.04-0.36). Significant correlations were also observed within NF2 families with various specific classes of mutations: truncating mutations--age at first symptom (0.41; 0.05-0.67) and number of meningiomas (0.09; 0.01-0.17); splice site mutations--age at first symptom (0.28; 0.04-0.49) and number of meningiomas (0.37; 0.13-0.61); and missense mutations--age at hearing loss (0.64; 0.16-0.88). Our findings are consistent with effects of both allelic and non-allelic familial factors on the clinical variability of NF2.

**The Cooperative Family Registry for Breast Cancer Studies: A Resource for Researchers.** *R.T. Senie<sup>1</sup>, I. Andrulis<sup>2</sup>, M. Daly<sup>3</sup>, J. Hopper<sup>4</sup>, S. Buys<sup>5</sup>, D. West<sup>6</sup>, H. Anton-Culver<sup>7</sup>.* 1) Sch Public Health, Columbia Univ, New York, NY; 2) Cancer Care Ontario, Toronto, Can; 3) Fox Chase Cancer Center, Cheltenham, PA; 4) Univ of Melbourne, Carlton, Aus; 5) Huntsman Cancer Center, Salt Lake City, UT; 6) Northern Calif. Cancer Canter, Union City, CA; 7) Univ of Calif, Irvine, CA.

The NCI funded the Cooperative Family Registry for Breast Cancer Studies [CFRBCS], three population-based and three clinic-based sites with an Informatics Support Center, providing a resource of family-based data and biospecimens for multidisciplinary gene-environment etiologic studies of familial breast and ovarian cancer. The participating 6,502 families [14,925 individuals] represent a broad spectrum of familial risk: a male with breast cancer, breast or ovarian cancer diagnosed <45 years, diagnosis with both cancers, or multiple relatives diagnosed with either cancer. Proband assist in recruiting maternal and paternal relatives, women and men with or without cancer. Participants provide pedigree information, health history, dietary data, and cancer treatment history on CFRBCS questionnaires. Blood and tumor tissue sections are banked at each site following rigid quality control procedures. Personal identifiers are removed assuring confidentiality; coded data are transmitted to the Informatics Center. BRCA1/2 founder mutations have been assessed in 1,417 Ashkenazi families [2,494 individuals]; 226 families with 336 mutation carriers [47 male and 289 females] were identified including one male with breast cancer and 191 females with breast or ovarian cancer. 130 carriers [36 males and 94 females] remain unaffected. Cancer and vital status are updated annually. During the 5 year renewal CFRBCS will expand minority family recruitment, increase participants per family, and maintain this invaluable resource for use by the research community at large. Studies using Registry resources, approved by the external Advisory Committee, are currently being conducted by CFRBCS investigators and international collaborators. Information about the CFRBCS and application procedures are available on the NCI website [www-dccps.ims.nci.nih.gov/CFRBCS](http://www-dccps.ims.nci.nih.gov/CFRBCS).

**Predictive heterozygote testing for multiple endocrine neoplasia type 1 (MEN 1) in Northern Finland. O.**

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MEN 1 is an autosomal dominant disorder with susceptibility to tumors of the parathyroid glands, endocrine pancreas or duodenum (GEP tumors), and anterior pituitary gland. We have ascertained 16 families with MEN 1 in Northern Finland with two founder mutations (1466del12 in 9 and 1657insC in 4 families) and two mutations (R527X and D418N) in singular families. In one family the diagnosis was done by linkage analysis. After genetic counseling the relatives at risk for MEN 1 were contacted by the counselees and those willing to participate received genetic counseling and possibility for heterozygote testing. The phenotypes of the observed heterozygotes were evaluated biochemically and radiologically. All patients and mutation carriers have been followed up by endocrinologists. Out of the 159 individuals tested 90 were found to be heterozygotes (84 mutation positive, 5 by linkage) including 26 probands or already known MEN 1 patients. Of the 81 patients with sufficient information 40 were regarded as healthy while 15 had been treated for one or more manifestations probably related to MEN 1. During the follow up 47 of the 55 previously undiagnosed heterozygotes were found to have primary hyperparathyroidism (PHPT), 25 had GEP-tumors, 14 had pituitary adenomas and 1 had a carcinoid tumor. Of the patients with PHPT 12 were treated surgically, as well as one of the patients with GEP- and carcinoid tumors, and one patient with a pituitary adenoma, respectively. Predictive testing of MEN 1 proved to be useful in the detection of the patients for early treatment. Genetic counseling including information about the pros and cons of the testing and careful follow up are essential in this process.

**Identification of a TNF-alpha nucleotide mutation in retinoblastoma patients.** *F. Pellestor<sup>1</sup>, I. Imbert<sup>1</sup>, D. Lohmann<sup>2</sup>, F. Munier<sup>3</sup>.* 1) Inst Genetique Humaine, CNRS, UPR 1142, Montpellier Cdx 5, France; 2) Inst Humangenetik, Essen, Germany; 3) CHU Vaudois, Lausanne, Switzerland.

Investigations on retinoblastoma with abnormalities on chromosome 6p have suggested that some genes on 6p could act in the tumor process. Interindividual variation in TNF-alpha expression has been described, indicating that the existence of functionally distinct TNF-alpha alleles could play a role in susceptibility to retinoblastoma. The search for heterogeneity within the TNF-alpha gene has disclosed several polymorphisms in the 5'UTR regulatory region which is crucial for the control of transcription. We have analyzed TNF-alpha gene sequence in 70 retinoblastoma patients. Ten of these retinoblastoma samples presented chromosomal abnormalities on 6p and have revealed an increase dosage of gene on 6p by prior CGH screening. These tumors were from patients with isolated unilateral retinoblastoma and in all tumors, two RB1 gene mutations were identified. Sequencing of the 5'UTR region of the TNF-alpha gene revealed a base substitution (T to C) at the position -28 as referred to mutation nomenclature. This single nucleotide mutation was present in 40 percent of the analyzed retinoblastoma tumors and was twice more observed in samples with chromosome 6p abnormalities. Since located in the regulatory region of the first exon of the TNF-alpha gene, this mutation does not disrupt the TNF-alpha protein sequence but could have implications for the level of TNF-alpha expression. This variation could modify the stability of secondary and tertiary mRNA structure and facilitate translation initiation, leading to an enhancement of TNF-alpha expression level in retinoblastoma tumors.

**Monoallelic expression of the paternal mutated allele in the pheochromocytoma of a patient with hereditary paraganglioma (PGL1) and a constitutional SDHD-gene mutation.** *A.S. Weinhaeusel<sup>1</sup>, P. Waldner<sup>1</sup>, R. Pfragner<sup>2</sup>, B. Niederle<sup>3</sup>, O.A. Haas<sup>1</sup>.* 1) CCRI, St Anna Children's Hosp, Vienna, Austria; 2) Inst. Path., University Graz, Austria; 3) Surgical Clinic, University Vienna, Austria.

Familial paraganglioma (PGL1; OMIM 168000) is a rare familial tumor predisposition syndrome. It results from mutations in the succinate-ubiquinone oxidoreductase subunit D gene (SDHD; Science 287:848;2000) that encodes the small subunit of cytochrome b in the mitochondrial complex II. Affected individuals develop slow growing tumors of the head and neck region, most commonly the carotid body. However, this is only the case, if the carriers inherit the mutation from their father. Although this pattern of inheritance is the classic clinical example of genomic imprinting, this phenomenon still lacks a molecular genetic explanation. We have detected a germ line missense mutation in the start codon of a patient with a paraganglioma and pheochromocytoma from a PGL1 family. This mutation enabled us to study the gene expression in the peripheral blood (PB) and in the corresponding pheochromocytoma tissue. In contrast to previous reports, we were unable to detect any SDHD m-RNA expression in the PB of either mutation carriers or normal controls. However, we experienced that due to the existence of several pseudogenes such expression studies are error-prone, in case DNA contamination cannot be excluded completely. In the tumor tissue, both alleles were present at the DNA level, whereas only the mutated paternal allele was expressed. Analysis of the methylation pattern in the 5'-CpG rich UTR of the SDHD gene with methylation-sensitive PCR did not reveal any allele-specific differences. Our preliminary observations provide a first glance on the potential mechanisms involved in the development of tumors in this tumor predisposition syndrome.

**Average risks of breast and ovarian cancer in women who carry a *BRCA1* or *BRCA2* mutation: a preliminary analysis of pooled family data from unselected case series.** P. Pharoah, A. Antoniou, H. Risch, S. Narod, J. Hopper, N. Loman, H. Olsson, O. Johansson, A. Borg, B. Pasini, P. Radici, D. Eccles, N. Tang, E. Olah, H. Anton-Culver, J. Eyfjord, D.G. Evans, C. Evans, J. Peto, D. Easton. BRCA1/2 Population Studies Collaborative Group.

Several methods have been used to estimate the ovarian and breast cancer risks for women who carry *BRCA1* or *BRCA2* mutations. Studies of multiple-case families have estimated risks to age 70 of 70-80%, while studies of families identified from unselected case series have estimated risks to age 70 of 40-60%. These differences may reflect the different sources of families, but the confidence limits have been wide. The purpose of this study was to collate family data from studies of *BRCA1/2* mutation prevalence in breast or ovarian cancer cases unselected for family history, in order to provide more precise estimates of average breast and ovarian cancer risks. So far, we have collected and analysed data from 13 studies that have screened a total of 4159 female breast cancer, 124 male breast cancer and 162 ovarian cancer cases for mutations in *BRCA1* and/or *BRCA2*. Family history data were available for 93 *BRCA1+* and 124 *BRCA2+* index cases. We used the pedigree information to estimate cumulative risks using a modified segregation analysis. The conditional likelihood of the pedigree was maximised given the genotype and phenotype of the index case. We parameterised the model in terms of log relative risks for mutation carriers compared to the general population in 10 year age groups. Non carriers were assumed to have the age-specific incidence of their population. The estimated cumulative risk of breast cancer in *BRCA1* mutation carriers was 41% (31-29) by age 50 and 64% (55-72) by age 70. The breast cancer risks for *BRCA2* mutation carriers at the same ages was 20% (12-27) and 49% (36-60). For ovarian cancer the risks by age 50 and 70 were 11% (7-18) and 37% (25-52) for *BRCA1* and 1% (0-1) and 20% (12-27) for *BRCA2*. These risks estimates are lower than those derived from selected multiple-case families. Analysis of a further four studies, which will almost double the number of mutation carriers, is in progress.

**Screening for founder mutations in BRCA1/2 tumor susceptibility genes among breast cancer families from North Sardinia.** *M. Pisano*<sup>1</sup>, *A. Cossu*<sup>2</sup>, *G. Palomba*<sup>1</sup>, *M.G. Sarobba*<sup>3</sup>, *A. Farris*<sup>3</sup>, *M.F. Dedola*<sup>4</sup>, *N. Olmeo*<sup>5</sup>, *A. Contu*<sup>5</sup>, *I. Persico*<sup>1</sup>, *M.P. Satta*<sup>1</sup>, *M. Ibbà*<sup>2</sup>, *M.R. Stratton*<sup>6</sup>, *M. Pirastu*<sup>1</sup>, *F. Tanda*<sup>2</sup>, *G. Palmieri*<sup>1</sup>. 1) Institute Molecular Genetics, Alghero, Italy; 2) Institute of Pathology, University of Sassari, Italy; 3) Dept. of Medical Oncology, University of Sassari; 4) Dept. of Radiotherapy, University of Sassari; 5) Dept. of Medical Oncology, A.S.L.1, Sassari; 6) Sanger Center, Cambridge, UK.

Genetically homogeneous Sardinian population can be helpful in defining the molecular basis of cancer. To evaluate the role of disease-causing mutations in breast cancer (BC), we screened tumor susceptibility genes BRCA1 and BRCA2 in cancer patients and their families from North Sardinia. Forty-seven BC families with at least 3 affected members, apparently unrelated and originated from the northern part of the island were selected. Mutation screening was performed on DNA from blood samples by a combination of techniques: haplotype analysis, DHPLC-based analysis, and automated sequencing. Among the BC families selected, only 3 presented association with ovarian cancer and in one of them we identified a new termination-codon mutation within the exon 11 of BRCA1 (L505Term). Two BRCA2 mutations, 8765delAG and I3412V, were detected in 6 and 1 (13% and 2%, respectively) families. Frequency of these mutations were then investigated in unselected BC patients from the same area of Sardinia: 8765delAG was found in 6/473 (1.3%) cases (all positive patients were from BC families with at least 3 cases), whereas none of the 61 BC cases analyzed presented the I3412V mutation. Altogether, 13 families with BRCA2 mutations were identified. Considering the total number of BC cases among the 53 families selected, BRCA2 mutations were registered in 7/13 (54%) families with 5 affected members, 4/17 (24%) with 4 BCs, and only 2/23 (9%) with 3 BCs. Although families studied here showed a clear predisposition to BC, majority of them have remained negative for mutations in BRCA1/2, suggesting that other mechanisms or genes are involved. However, BRCA2 mutations seem to be strongly correlated to breast cancer development among Sardinian families with high recurrence of cases.



**CHK2 in familial breast cancer.** *P. Vahteristo*<sup>1</sup>, *J. Bartek*<sup>2</sup>, *J. Bartkova*<sup>2</sup>, *S. Ojala*<sup>1</sup>, *H. Eerola*<sup>3</sup>, *J. Kononen*<sup>4</sup>, *P. Heikkila*<sup>5</sup>, *OP. Kallioniemi*<sup>4</sup>, *H. Nevanlinna*<sup>1</sup>. 1) Obstetrics and Gynecology, Helsinki University Central Hospital, Helsinki, Finland; 2) Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark; 3) Department of Oncology, Helsinki University Central Hospital, Helsinki, Finland; 4) Cancer Genetics Branch, NHGRI/NIH, Bethesda, MD; 5) Department of Pathology, Helsinki University Central Hospital, Helsinki, Finland.

CHK2 is a cell cycle checkpoint kinase that phosphorylates p53 in response to DNA damage. p53 also reciprocally downregulates CHK2, indicating that p53 and CHK2 may play interdependent and complementary roles in cell cycle regulation after DNA damage. Rare germline mutations in CHK2 have been found in families with Li-Fraumeni syndrome (LFS) or phenotypically suggestive of LFS. Previously, we found two CHK2 positive kindreds originally recruited through breast cancer patients. Here, we have further evaluated the role of CHK2 in hereditary breast cancer by analyzing the CHK2 expression on breast tumors from breast cancer families. A mouse monoclonal anti-CHK2-antibody was used in the immunohistochemical staining of a tumor micro-array including 186 breast carcinomas from 20 BRCA1/2 positive and 76 negative kindreds. In six tumors (five BRCA1/2 negative and one BRCA2 positive), grossly aberrant CHK2 expression was observed: both the staining intensity and the number of positive cells were reduced. In these six families, germline mutations in the CHK2 gene were searched for, and in one family a protein truncating mutation was found in exon 10 (1100delC). The proband was diagnosed with breast cancer at 41 years, and her mutation was inherited from the father diagnosed with prostate cancer at 76 years. In the maternal lineage, a BRCA1 mutation segregates in the family, and the proband's sister (breast cancer at 48 years) is a double heterozygote for both mutations. The same 1100delC CHK2 mutation has previously been found in one family by Bell et al. (1999) and by us now in three families, indicating this site as a mutational hot spot. Further analyses are under way to evaluate the frequency and clinical significance of the 1100delC-mutation in a larger sample material of breast cancer families and unselected breast cancer patients.

**Selection of Chromosome 11p Loss in the tumorigenesis of VHL-related Pheochromocytoma.** *B.T. Teh<sup>1</sup>, W.O. Lui<sup>2</sup>, J.D. Chen<sup>1</sup>, S. Glasker<sup>4</sup>, E. Kort<sup>3</sup>, C. Larsson<sup>2</sup>, H.P.H. Neumann<sup>4</sup>.* 1) Lab Cancer Genetics, Van Andel Research Inst, Grand Rapids, MI; 2) Department of Molecular Molecular Medicine, Karolinska Hospital, Sweden; 3) Laboratory of Analytical Cellular and Molecular Microscopy, Van Andel Research Inst, Grand Rapids, MI; 4) Department of Nephrology and Hypertension, Albert-Ludwigs-University, Freiburg, Germany.

By using comparative genomic hybridization (CGH), we characterized the genetic profiles of 36 VHL-related pheochromocytomas. We then compared the results with sporadic and MEN2-related pheochromocytomas, which are either previously reported or included in the present study. In 36 VHL-related tumors, loss of chromosome 3 and chromosome 11p were found in 34 tumors (94%) and 31 tumors (86%), respectively. There was significant concordance of deletions in chromosomes 3 and 11p ( $Kappa = 0.64$ ,  $p=0.0095$ ), and chromosome 11p deletions were only found in tumors exhibiting chromosome 3 deletions, suggesting that they are involved in the same genetic pathway in VHL-related pheochromocytoma. The loss of chromosome 11p appears to be VHL-specific and pheochromocytoma-specific. It is not present in any of the 10 studied cases of VHL-related CNS hemangioblastomas and is significantly less common in sporadic pheochromocytomas (13%;  $p<0.0001$ ) and MEN2-related pheochromocytomas (30%;  $p=0.0012$ ). In conclusion, we identified a novel genetic abnormality (i.e., loss of chromosome 11p), which coupled with the VHL mutation, is important in the tumorigenesis of VHL-related pheochromocytoma.

**cDNA array analysis of NF1 plexiform tumor Schwann cell cultures.** *S.A.M. Thomson<sup>1</sup>, L. Fishbein<sup>1</sup>, D. Muir<sup>2</sup>, M.R. Wallace<sup>1</sup>.* 1) Molecular Genetics and Pediatric Genetics, Univ. of Florida, Gainesville, FL; 2) Pediatric Neurology, and Neuroscience, Univ. of Florida, Gainesville, FL.

Neurofibromatosis 1 (NF1) is a common dominant autosomal disease occurring in about 1 out of 3500 births worldwide. The NF1 gene produces a transcript of 11-13 kb that encodes the tumor suppressor neurofibromin. The hallmark feature of NF1 is the neurofibroma, a benign fibromatous nerve sheath tumors containing primarily Schwann cells. Dermal neurofibromas are small and arise along peripheral nerve twigs. Plexiform tumors are often much larger, arising from more deeply placed peripheral nerves. Plexiforms and spinal neurofibromas can cause considerable medical problems, and there are no treatments other than surgery (often inadequate). Malignant peripheral nerve sheath tumors (MPNST's) occur in about 5% of NF1 patients and arise in pre-existing plexiforms. Neurofibromin is reduced/absent in a population of tumor Schwann cells (due to somatic mutation) and is thus likely a common first step in tumorigenesis. Little is known about whether loci other than NF1 are altered in plexiform neurofibromas. To address this, we compared RNA from plexiform tumor and normal Schwann cell cultures using the Atlas Human Cancer cDNA arrays. Our study included 9 plexiform tumor cell cultures and one normal NF1 nerve Schwann cell culture. Overall, the expression patterns of the tumor cultures were similar to that of normal Schwann cell cultures, although there was some heterogeneity. About a dozen transcripts (e.g. TIMP3, MMP11) had consistently elevated or decreased levels in the majority of samples. In addition, we compared 4 plexiform tumor cell cultures and one normal Schwann cell culture using the Human Cancer Affymetrix chips. Several genes are differentially expressed in both types of arrays, and additional genes have been identified utilizing the Affymetrix system. These differences are being confirmed with real-time PCR and these data and discussion will be presented. Identification of commonly-altered genes may lead to improved understanding of NF1 signaling, sub-classification of plexiform tumors, and identification of therapeutic targets.

**Wild-type BRCA2 is not sufficient to complement DNA damage repair deficiency of Capan-1 cell line.** *L.-K. Su.*  
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People carrying a mutant allele of *BRCA2* have increased risk for breast, ovarian, pancreatic and other types of tumors. *BRCA2* has been suggested to play an important role in repairing double strand DNA breaks (DSB) based on several observations. One is that *BRCA2* interacts with *RAD51*, an evolutionary conserved protein important for repairing DSB. The other is that mouse cells deficient of *Brca2* are highly sensitive to DNA damaging agents and have aberrant chromosomes. In addition, Capan-1 cell line has been shown to be more sensitive to DNA damaging agents and deficient in repairing DSB comparing to other human cell lines. Capan-1 is a human pancreatic cancer cell line and is the only human cell line known to not express wild-type *BRCA2*. The increased sensitivity of Capan-1 to DNA damage has been attributed to its lack of wild-type *BRCA2*.

I have been investigating whether expression of the wild-type *BRCA2* is sufficient to reduce the sensitivity of Capan-1 cells to DSB. I have generated two Capan-1 derivatives that constitutively express the wild-type *BRCA2* and another one that expresses the wild-type *BRCA2* under the regulation of tetracycline. I compared the sensitivity to g-radiation of the two constitutive *BRCA2*-expressing derivatives to that of the Capan-1, there was no detectable difference. I also compared the sensitivity to g-radiation of the regulated *BRCA2*-expressing Capan-1 derivative between when it expressed the wild-type *BRCA2* and when it did not express the wild-type *BRCA2*. Again, there was no detectable difference. These results suggest that expression of the wild-type *BRCA2* alone is not sufficient to reduce the sensitivity of Capan-1 cells to DSB. I am currently examining the sensitivity of these cells to DNA damaging chemicals and the ability of *RAD-51* in these cells to form g-radiation induced foci. My results will clarify the question of whether the difference in the sensitivity to DSB between Capan-1 and other human cell lines is only due to the lack of wild-type *BRCA2* in Capan-1 cells.

**A Novel High-Density Oligonucleotide Array for Hereditary Non-Polyposis Colon Cancer.** *D. Shanmugarajah*<sup>1</sup>, *S.M. Lipkin*<sup>2</sup>, *J.G. Hacia*<sup>3</sup>, *N. Hunt*<sup>4</sup>, *S.P.A. Fodor*<sup>4</sup>, *F.S. Collins*<sup>1</sup>. 1) Genetics and Molecular Biology, NHGRI/NIH, Bethesda, MD; 2) Dept of Medicine, University of California, Irvine, CA; 3) University of Southern California, Los Angeles, CA; 4) Affymetrix Corporation, Santa Clara, CA.

Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is a highly penetrant, autosomal dominant syndrome characterized by early-onset colorectal, uterine, ovarian, renal, and small bowel cancer. Germline mutations in five mismatch repair genes (MSH2, MLH1, PMS1, PMS2 and MSH6) have been identified in families with HNPCC. Technical limitations associated with current mutational screening of candidate genes have placed increased emphasis on the development of new technologies to detect sequence changes underlying disease, especially when the candidate genes are large and all possible coding region changes must be found. High density oligonucleotide arrays (DNA chips) offer an attractive alternative to direct sequencing, and have been used previously to detect mutations in the BRCA1, BRCA2 and ATM genes. In this study we describe the use of DNA chips to screen MLH1, MSH2 and MSH6 for all possible sequence variations (HNPCC DNA Chip). These arrays consist of 25-mer oligos synthesized on a silicon chip. A total of 250,000 oligonucleotide features contain the complete MLH1, MSH2 and MSH6 sense and antisense coding sequences (>11kb). All single bp substitutions, deletions of 1-5bp, and 100 known mutations are tiled on the chips as well. We are currently determining the test operating characteristics of the HNPCC DNA Chip on patient samples with known mutations to determine sensitivity and specificity for mutation detection using this novel technology. In preliminary studies, 17 samples with known mutations have been tested, and 15 mutations (88%) were found. The chips are now being applied to look for HNPCC mutations in affected individuals with a family history of colon cancer.

**A novel APC splice mutation associated with attenuated FAP.** *J.L. Scalia<sup>1</sup>, I. Tepler<sup>1</sup>, P.L. Weinstein<sup>1</sup>, V.M. Pratt<sup>2</sup>, K. Snow<sup>3</sup>.* 1) Cancer Risk and Prevention Program, Stamford Hosp, Stamford, CT; 2) LabCorp, RTP, NC; 3) Molecular Genetics Laboratory, Mayo Clinic, Rochester, MN.

Adenomatous polyposis coli (APC) germline mutations are detected in an estimated 80 to 90% of familial adenomatous polyposis (FAP) kindreds. The detection rate for attenuated FAP (aFAP) is unclear but is definitely lower than that for classical FAP. Attenuated FAP is typically associated with later onset of fewer polyps and absence of extracolonic manifestations. We report a kindred (TSH-77-98) in which the affected family member presented with multiple polyps at age 42, without familial evidence of disease. Mutation screening by CSGE and DNA sequencing demonstrated the DNA alteration c.1548G>A, which is at the last nucleotide of exon 11. The affected codon is unchanged (L516L). Using the Neural Network Splice Site Predictor, c.1548G>A results in a decreased splice donor score from 1.00 to 0.84. The Gene Feature Search program FEXH showed a decrease in donor site probability from  $w=8.60$  to  $w=3.72$ . Using the program FGENESH, c.1548G>A caused the donor site to be not predicted (from a score of 14.94 for the donor site in wild-type sequence). Protein truncation testing (PTT) of APC segment 1 (performed twice using 2 blood specimens) identified a truncated product at 55kDa. A truncated RT-PCR product for segment 1 showed skipping of exons 2 to 14 although sequencing of genomic DNA did not identify any alterations in exon 1 or adjacent intronic sequence. The 55kDa protein product may have been derived from another slightly truncated RT-PCR product that could not be adequately resolved from the normal product to allow sequence analysis. Our findings suggest that the novel exon 11 alteration is a de novo deleterious mutation causing late-onset FAP. The atypical presentation supports previous associations between 5APC mutations and aFAP. This case also demonstrates the value of PTT in confirming the significance of equivocal germline APC sequence alterations.

**Common colon neoplasia is not linked to germline APC variants.** *G.L. Wiesner*<sup>1,2,4</sup>, *D. Daley*<sup>3</sup>, *P. Platzer*<sup>4,5</sup>, *H. Yan*<sup>5,6</sup>, *C. Ticknor*<sup>4</sup>, *R. Elston*<sup>3</sup>, *B. Vogelstein*<sup>5,6</sup>, *S. Markowitz*<sup>4,5</sup>. 1) Center for Human Genetics, University Hospitals of Cleveland; 2) Department of Genetics, Case Western Reserve University; 3) Department of Epidemiology, Case Western Reserve University; 4) Ireland Cancer Center, University Hospitals of Cleveland, Case Western Reserve University, Cleveland, OH; 5) Howard Hughes Medical Institute; 6) Oncology Center, Johns Hopkins Medical Institutions, Baltimore, MD.

First degree relatives of individuals with colon adenomatous polyps or CRC demonstrate a 2 to 3 fold risk for CRC, suggesting a genetic basis to CRC susceptibility in seemingly "sporadic" colon cancer. Recently, the carriage of the I1307K variant in the APC gene has been associated with a 2-3 fold increased risk of CRC in the Ashkenazi Jewish population, suggesting that "mild" variant alleles in the APC gene might act more generally as susceptibility genes accounting for a proportion of colon neoplasia in the general population. We tested this hypothesis by performing an affected sibling pair linkage analysis of the APC locus on 116 kindreds enrolled in the CWRU Colon Neoplasia Sibling Study (CNSS). Kindreds that conformed to diagnostic criteria for FAP and HNPCC were excluded from the analysis. 164 sibling pairs with pathologically verified CRC or adenomatous polyps were genotyped for two polymorphic markers (D5S82 and D5S346) flanking the APC locus. Preliminary results found no evidence for linkage with using a single point model-free linkage analysis (estimate of allele sharing =0.507; S.E.=0.0244; P value =0.38). Further, the transmission disequilibrium test (TDT) and Haseman-Elston regression analysis yielded non-significant results. While additional markers in the region are currently being genotyped to refine the estimate of allele sharing in these affected sibpairs, our results suggest that common forms of CRC are unlikely to be caused by variant alleles of the APC gene segregating in the general population.

**Antisense inhibition of methylenetetrahydrofolate reductase reduces cell survival of methionine-dependent colon carcinoma cells *in vitro*.** *J. Sekhon<sup>1</sup>, P. Pereira<sup>1</sup>, A.R. Schievella<sup>2</sup>, R. Rozen<sup>1</sup>.* 1) Departments of Human Genetics, Pediatrics and Biology, McGill University, Montreal, QC, Canada; 2) Variagenics Inc., Cambridge, MA.

Many diverse cancer cell types have been shown to be methionine (Met)-dependent. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF is a co-substrate for homocysteine remethylation to Met catalyzed by the vitamin B12-dependent enzyme Met synthase. This study aimed to determine if MTHFR is critical for Met synthesis and if MTHFR downregulation in transformed lines reduces cell survival. First, we examined the growth of normal fibroblasts, of MTHFR-deficient fibroblasts (WG 1554) and of colon carcinoma lines in Met-deficient medium (M-), in M- containing homocysteine and vitamin B12 (M-H+), and in replete medium. Second, to test the effects of decreased MTHFR levels, we transfected antisense oligonucleotides (ASOs) complementary to the MTHFR mRNA into the Met-dependent colon carcinoma cell line SW620. All tested cell lines showed sensitivity to M-, demonstrating that Met is needed to sustain cell growth. WG 1554 fibroblasts, with zero MTHFR activity, were unable to maintain cell growth in M-H+, likely due to the inability to synthesize 5-MTHF. Four colon carcinoma cell lines increased proliferation only slightly in M-H+, yet the growth of two normal fibroblast lines in M-H+ approached levels obtained in replete MEM, demonstrating the increased Met requirement of these cancer cell lines. To decrease MTHFR levels, two ASOs, 677T and EX5, were tested. SW620 cells treated with 400 nM of 677T decreased cell survival by approximately 70% ( $p < 0.0001$ ), and treatment with 400 nM of EX5 decreased cell survival by approximately 80% ( $p < 0.01$ ), compared to control ASO treated cells. MTHFR activity decreased by 80% after treatment with 677T, compared to the activity of control ASO treated cells ( $p < 0.01$ ). Western blot analysis showed a decrease in MTHFR protein levels after 677T or EX5 treatment. This study supports the notion that MTHFR inhibition by antisense or by other means might be useful in cancer treatment.



**The molecular genetic basis of multiple meningiomas.** *B. Heinrich, C. Hartmann, M. MacCollin.* Department of Neurology, Massachusetts General Hospital, Charlestown, MA 02129, USA.

Meningiomas are common tumors of the linings of the central nervous system, comprising one fifth of all intracranial neoplasms. Between 1 and 8% of patients with meningioma develop multiple meningiomas, a trait rarely reported to be transmitted in an autosomal dominant fashion. The only loci associated with sporadic meningioma are *NF2* on chromosome 22, and the related cytoskeletal element *DAL-1* on chromosome 18. We investigated these two loci in multiple meningioma patients. Seven multiple meningioma patients without evidence of *NF2* were identified; three had affected relatives. Unexpectedly, all affected individuals and their affected relatives were female. Exon scanning was used to search for alterations in the *NF2* and the *DAL-1* genes in genomic samples from tumor and blood specimens. Loss of heterozygosity (LOH) analysis was performed using intragenic and flanking markers at both loci. Truncating *NF2* mutations were detected in three tumor specimens, but were not present in the corresponding blood samples. Two tumors showed LOH at the *NF2* locus. All tumors showing alteration (mutation or LOH) at the *NF2* locus were from sporadically affected patients. *DAL-1* gene analysis revealed 3 missense alterations, 1 silent mutation in a codon wobble position and 1 change in the non-conserved region of intron 9. In contrast to the *NF2* results, all exonic alterations were found in paired blood specimens. LOH for chromosome 18 was not seen in any of these tumors. These results suggest that the molecular basis of sporadic and familial multiple meningiomas is fundamentally different, with *NF2* inactivation contributing significantly to the former condition. Non-truncating alterations in the *DAL-1* gene were common in this small cohort. Their contribution to predisposition to tumor formation is under study.

***MLH1* promoter hypermethylation in endometrial carcinoma.** *L.H. Honore*<sup>1</sup>, *C. Otto*<sup>2</sup>, *K. Helmle*<sup>2</sup>, *D. Whiteside*<sup>2</sup>, *S.E. Andrew*<sup>2</sup>. 1) Dept Pathology, Univ Alberta, Edmonton, AB, Canada; 2) Department of Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Microsatellite instability (MSI+), resulting from a lack of DNA mismatch repair, is observed in approximately 20% of sporadic endometrial tumours and over 90% of the endometrial cancers arising in hereditary non-polyposis colorectal cancer patients. Despite a lack of mismatch repair, less than 10% of MSI+ endometrial tumours have known mutations in *MSH2* or *MLH1*, the two major mismatch repair genes. It has been shown that promoter hypermethylation contributes to the inactivation of *MLH1* in such MSI+ mutation-negative tumours by silencing of transcription from the *MLH1* promoter. However, the particular sites that are critical for *MLH1* silencing are not known. We used the bisulfite sequencing assay, to study the methylation status of 44 CpG sites within the *MLH1* promoter region (spanning the region 226 to -724 bp upstream of the ATG) of 19 endometrioid tumours. Over 84% (16/19) of the MSI+ endometrioid tumours are completely or nearly completely methylated throughout the region examined. Three of 19 MSI+ and 5 of 7 MSI stable samples have large unmethylated regions, perhaps decreasing the level of methylation-mediated repression of the promoter. However, MSI stable samples with methylated sites as well as some unmethylated CpG sites within otherwise highly methylated regions in MMR deficient samples highlights the importance of using assays that assess CpG sites that are important for controlling gene expression for epigenetic analysis. From preliminary results, regions closer to the coding region appear to have a greater influence the microsatellite status of the tumour, likely through controlling the expression of *MLH1*. Thus, it is important to gain an overall understanding of the methylation status of the entire promoter in relation to gene expression, as methylation patterns change between regions.

**Molecular characterisation and identification of MEN1 gene mutations in families with familial isolated hyperparathyroidism(FIHP).** A. Villablanca<sup>1</sup>, WS. Wassif<sup>2</sup>, K. Jones<sup>2</sup>, M. Kassem<sup>3</sup>, T. Carling<sup>5</sup>, P. Harris<sup>4</sup>, O.

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Familial isolated hyperparathyroidism (FIHP) is defined as hereditary primary hyperparathyroidism without association with other diseases or tumours. To date, FIHP has been defined to exist as a variant of other familial tumour syndrome in which primary hyperparathyroidism is the main feature, e.g. multiple endocrine neoplasia (MEN 1) and the hyperparathyroidism jaw-tumour syndrome (HPT-JT). Here we describe a subset of unreported families in an attempt to investigate the genetic background of FIHP. The entire MEN1 gene was sequenced for germline mutations and in addition tumour specimens were analysed using comparative genomic hybridisation (CGH) and loss of heterozygosity (LOH) studies. Genotyping and linkage analysis was carried out with microsatellite markers in the MEN1 locus and for the suggested HPT-JT region. In this study, we found germline mutations of the MEN1 gene, segregating with primary hyperparathyroidism. Copy number changes detected in the families with germline mutations included loss of chromosome 11. Other alterations discovered was loss of chromosome 15, 16 and gain of X. These results suggest that it is likely that the detected germline mutations would result in a non-functional menin protein causing a familial predisposition to cell proliferation in the parathyroid gland, consistent with a tumour suppressor mechanism. We conclude that the altered MEN1 gene function is of importance in the development and genesis of FIHP.

**Characterization of the chimeric erythropoietin receptor fusion protein in the TF-1 erythroleukemia cell line.**

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Erythroleukemia is a rare hematologic malignancy that carries a poor prognosis. The role of the Erythropoietin Receptor (EpoR) in the pathogenesis of erythroleukemia has been an area of great interest. In the human erythroleukemia cell line, TF-1, a chromosome rearrangement was discovered at the EpoR locus, 19p13.3 (Ward et al, 1992, *Exp Hematol*; 20:371). The rearrangement deletes the 3'end of the EpoR gene and replaces it with a novel sequence that we call the EpoR-fusion partner. The abnormal EpoR fusion partner chimera in TF-1 cells produces a highly expressed message which is translated into a protein with deletion of the C-terminus of the EpoR. In order to understand the biological significance of the EpoR-fusion partner chimera, we transfected 32D cells, an immortalized growth factor dependent murine myeloid cell line, with cDNA constructs encoding the abnormal EpoR fusion partner chimera (EpoRTF-1) and an EpoR that is truncated at the site of the translocation (EpoRT). We assayed proliferation rates of the transfected cells growing in Epo. We observed that EpoRTF-1 and EpoRT cells proliferate at similar rates and that the EpoRTF-1 construct does not confer growth factor independence. We also obtained a BAC clone containing the fusion partner and mapped it to chromosome 8 by fluorescent-in-situ hybridization. Using this BAC clone we assembled a contig containing the EpoR fusion partner gene and analyzed the sequence using bioinformatic tools. Our results show a predicted gene in our sequence which matches a mouse protein secreted from lymphocytes. We are currently cloning the human homologue. Our results show that one copy of the EpoR locus in TF-1 cells has undergone a translocation between chromosome 19 and chromosome 8 and that this translocation may take place within a novel human gene.

**Molecular evidences that recurrent breakage at the common fragile site FRA7G, might lead to amplification of the MET oncogene in a human carcinoma.** A. Hellman<sup>1</sup>, E. Zlotorynski<sup>1</sup>, S.W. Scherer<sup>2</sup>, J. Skaug<sup>2</sup>, B. Kerem<sup>1</sup>. 1) Dept Genetics, Hebrew Univ Jerus, Jerusalem, Israel; 2) 1Department of Genetics, The Hospital for Sick Children, Ontario, Canada.

Specific chromosomal structures known as 'common fragile sites' (CFS) are prone to breakage and rearrangements in culture cells. In rodent model for oncogene amplification, the cytogenetic locations of CFS coincided with recurrent chromosomal breaks that drove breakage-fusion-bridge (BFB) cycles, leading to intra-chromosomal amplification (ICA) of selected genes. Whether BFB cycles are underlying ICA of human oncogenes, and whether CFS breaks drive these amplifications, awaited molecular analysis of amplicons and of their adjacent CFS. Here we study the structure of MET ICA in the GTL-16 cells, deriving from a human gastric carcinoma. Our analysis showed that two large amplicons (~8 and ~10 Mb), both containing MET, are organized in a head-to-head structure and in equal-spaced intervals. This organization fits well with the predictions of the BFB model. We further mapped two chromosomal breakpoints, which defined the centromeric boundaries of these amplicons. Next, we characterized the regions which harbors and flanks the CFS, FRA7G. We found two distinct regions within FRA7G: one encompassed ~700 kb including MET, comprised FISH probes that spanned the cytogenetic appearance of FRA7G. This region replicated at mid-S phase and exhibited intrinsic replication perturbation, as previously found in another CFS, FRA7H. The other FRA7G region, encompassed at least 2.8 Mb including the centromeric boundaries of the GTL-16 amplicons, comprised clones that hybridized only to the telomeric side of FRA7G, although these clones lie on the centromeric side of the physical map. We showed that this is not the result of genomic rearrangements, thus might reflect the unusual chromatin organization at active (exhibited) CFS. Our findings suggests that activation of common fragile sites might lead to amplification of oncogenes, as well as to other chromosomal rearrangements, thus may play an important role in both the generation of tumor cells and in their evolution towards increasingly malignant phenotypes.

**Detecting gene amplification in breast cancer by combining Gene expression profiling and Gene mapping.** *M.H. Polymeropoulos<sup>1</sup>, A. Baras<sup>1</sup>, T.M. Walz<sup>2</sup>, I. Kwon<sup>1</sup>, M.A. Dressman<sup>1</sup>.* 1) Pharmacogenetics, Novartis Pharmaceuticals Corporation, Gaithersburg, MD; 2) Department of Biomedicine and Surgery, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden.

Global gene expression analysis using microarrays has been used to characterize the molecular profile of tumors. Gene expression variability at the mRNA level can be caused by a number of different events, including novel signaling, downstream activation of transcription enhancers or silencers, somatic mutation and genetic amplification or deletion. Gene amplifications are commonly observed in cancer and often include known oncogenes. The tyrosine kinase-type cell surface receptor, ERBB2, is an oncogene located on chromosome 17q22. ERBB2 is amplified in 25-30% of breast tumors. We report here two genes, PNMT and MLN64, which are co-expressed in 45 breast cancer biopsies with ERBB2 and also map within the same chromosomal location. Co-expression and co-localization of PNMT, MLN64 with ERBB2 suggests that the amplification of ERBB2 includes the chromosomal region that harbour these genes. This approach of combining Gene Expression profiling and gene Mapping (GEM) can be further exploited for the identification of gene deletions or amplifications.

**Multiple fragile sites are deleted in cancer cells.** *M.F. Arlt<sup>1</sup>, D.G. Beer<sup>2</sup>, T.W. Glover<sup>1</sup>*. 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Surgery, University of Michigan, Ann Arbor, MI.

The colocalization of common fragile sites and translocation breakpoints has suggested that fragile sites play a role in genomic rearrangements seen in cancer. We investigated five common fragile sites (FRA3B, FRAXB, FRA7G, FRA7H, and FRA16D) for deletions in 21 cancer cell lines and 6 primary esophageal adenocarcinomas. PCR analysis revealed FRA3B to be the most frequently deleted fragile site with 6/27 (22.2%) showing homozygous deletions. 14.8% (4/27) of the samples were characterized by deletions at FRAXB. 11.1% (3/27) of the samples had homozygous deletions at FRA16D. FRA7G and FRA7H each had homozygous deletions in one tumor (3.7%). Overall, one third (9/27) of the specimens were characterized by at least one fragile site deletion. 11.1% (3/27) of the samples had deletions at more than one fragile site. None of the samples had deletions at any of the six control markers analyzed. Also, deletions were mostly restricted to within the fragile sites tested, with only one tumor (3.7%) showing deletions of sequences immediately flanking FRAXB and FRA16D. In addition, each fragile site was deleted in at least one tumor or cell line. PCR analysis revealed two cell lines with apparently identical deletion breakpoints that colocalize with the boundaries of FRAXB at DXS1130 and DXS1133 on Xp22.3. One of these cell lines, BIC-1, was treated with aphidicolin and found to lack gaps and breaks at FRAXB in 100 metaphases examined, indicating that the deletion eliminated sequence necessary for fragility. The two primary tumors with FRAXB deletions also share the same proximal deletion boundary with these two cell lines. The colocalization of fragile site boundaries and deletion breakpoints suggests that fragile sites are mechanistically involved in the deletion process in these regions. We have shown that deletions occur at multiple common fragile sites, including FRAXB and FRA3B, suggesting that instability at fragile sites is a general phenomenon in some tumors.

**Genome-wide screening of genes associated with chemosensitivity of human cancer xenografts by means of cDNA**

**microarray.** *H. Zembutsu<sup>1,5</sup>, T. Tsunoda<sup>2</sup>, Y. Furukawa<sup>1</sup>, Y. Ohnishi<sup>3</sup>, T. Katagiri<sup>1</sup>, J. Okutsu<sup>1</sup>, Y. Ueyama<sup>4</sup>, N.*

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One of the most serious concerns in present cancer therapy is that we have no appropriate way to predict the sensitivity or toxicity to anticancer drugs. Hence, establishment of the predictive method is an urgent issue to be solved. In order to identify genes possibly associated with chemosensitivities to nine different anticancer drugs, we analyzed expression profiles of a panel of 85 human cancer xenografts derived from nine organs by means of cDNA microarray representing 23,040 genes. The human cancer xenografts were implanted into nude mice, and their chemosensitivities to anti-cancer drugs including 5FU, ACNU, ADR, CPM, DDP, MMC, MTX, VCR, and VLB were measured. Hierarchical cluster analysis using expression profiles of the 23,040 genes categorized the xenografts approximately into groups of organ from which they were established. We compared the expression profiles with chemosensitivities to each anti-cancer drug, and identified 1578 genes significantly correlated to the chemosensitivities. These data provide important information for identification of useful predictive markers for drug sensitivity, for the selection of personalized chemotherapy with effective anti-cancer drugs, and for improvement of their effect by modulating genes associated with drug sensitivity.



**Survival after radical radiotherapy for prostate cancer in a UK series of familial and sporadic cases. A.**

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Prostate cancer is a major public health problem. Approximately 13000 cases are diagnosed each year in the UK, and in the US it is the commonest malignancy in men, with over 180000 new cases expected to be diagnosed in 2000. Studies have shown that the risk of developing prostate cancer is increased 2-3 fold in the first degree relatives of cases. Other reports have indicated that familial cases have earlier onset compared to sporadic cases, but there is concern about the confounding effect of PSA screening. However no difference in survival between the two groups has been reported. A 20 year series of men with organ-confined and largely clinically detected prostate cancer treated with radical radiotherapy at the Royal Marsden Hospital further supports this finding. 1036 men were treated, of whom 107(9.7%) had a family history of prostate cancer. The 5-year overall survival was 68% in sporadic cases and 76% in familial cases (HR=0.77, 95%CI 0.52-1.03). Disease specific survival will be presented. No statistically significant difference was found in overall survival in sporadic vs. familial cases in this group of UK patients. Funded by National Lottery Charities Board.

**Prophylactic Bilateral Oophorectomy and Breast Cancer Risk Reduction: A Pilot Study.** *J.E. Olson, S.J. Iturria, J.L. Hernandez, L.C. Hartmann, T.A. Sellers.* Mayo Clinic Cancer Center, Rochester, MN.

**Background:** Estrogens are clearly implicated in the pathogenesis of breast cancer. The ovaries are the primary source of circulating estrogens in premenopausal women. Removal of the ovaries has been shown to reduce risk of breast cancer, but the benefit of this procedure in women with familial risk is not well established. **Objective:** To investigate whether removal of the ovaries is an appropriate clinical management tool for women at high or moderate risk of breast cancer (BrCa) based on family history of breast cancer. A pilot study was conducted to determine the feasibility of creating an oophorectomy cohort at Mayo Clinic. **Methods:** Women were identified from the Mayo Clinic Surgical Index whose surgical code indicated a possible bilateral oophorectomy during 1970 - 94 and who were less than age 60 at time of surgery. A random selection of charts (n=2272) was reviewed from all even-numbered years until at least 50 eligible women were identified from each year. Information was collected via mailed questionnaires with telephone follow-up of non-responders. **Results:** Of 852 eligible women, 680 women (80 percent) provided data (645 self and 35 surrogate respondents), 7 percent refused, 2 percent were deceased, 11 percent could not be located or were unable to participate for miscellaneous reasons. Women were grouped into low risk (no BrCa in 1st or 2nd degree relatives, N=237) and moderate/high risk (M/H, 1 or more relatives with BrCa, N=248). Based upon 1978-98 SEER rates (low risk) and the Gail model (M/H), 16.2 cases of BrCa were expected in M/H women, 12 were observed (p=0.25). In low risk women, 10.3 BrCa cases were expected, 5 were observed (p=0.05). Among women with surgery before age 51, there was an even more pronounced difference between the observed and expected numbers of cancers. In M/H women, 11.6 cases expected, 6 were observed (p=0.05); 6.9 cases were expected and 2 were observed in low risk women (p=0.01). **Conclusion:** In these pilot data, oophorectomy was associated with a reduction in BrCa in both M/H and low risk women. These results need to be confirmed within the entire cohort of women.

**Knowledge, Awareness, Intentions and Decisions Regarding Genetic Testing for HNPCC.** *D. Hadley<sup>1</sup>, J. Jenkins<sup>2</sup>, E. Dimond<sup>2</sup>, D. Liewehr<sup>3</sup>, S. Steinberg<sup>3</sup>, I. Kirsch<sup>2</sup>.* 1) NHGRI, NIH, Bethesda, MD; 2) NCI, NIH, Bethesda, MD; 3) NCI, Biostatistics, Bethesda, MD.

The application of genetic testing to refine cancer risks is most often discussed in the context of families with inherited breast/ovarian cancers. We seek to understand factors that affect decisions regarding genetic testing in families with Hereditary Nonpolyposis Colorectal Cancer (HNPCC). Baseline responses by the first 104 participants focusing on awareness, knowledge, intentions, and decisions regarding genetic testing for HNPCC are presented.

Adults with colon cancer (MSI+) and a family history suggestive of HNPCC complete a baseline questionnaire assessing demographic and psychological variables along with cancer screening practices. Participants are then provided a structured education session followed by counseling and the option of genetic testing. Psychological and behavioral outcomes are reassessed at 6 and 12 months. For those choosing testing, results and supportive counseling occur in person. First-degree adult relatives of probands with identified HNPCC germ-line mutations are offered participation.

Of the one hundred sixty-four (164) persons identified as eligible, one hundred four (104) elected to participate (63%). As baseline, sixty-four percent (64%) of participants had heard "almost nothing" or "relatively little" about genetic testing for colon cancer. Despite this relative lack of information, a majority of participants (97%) stated intentions (probably or definitely) to pursue genetic testing prior to education and counseling. The majority of participants (51%) identified learning about their children's risks of developing cancer as the most important reason to consider genetic testing. Thirty-nine percent (39%) identified concerns regarding the potential impact on insurance coverage as the most important reason to not undergo genetic testing. Fifty-one percent (51%) of eligible family members elected to pursue genetic testing. Of those opting to participate in the study, 96% elected to undergo testing. Further descriptive and statistical data will be presented.

**Online disease-specific variant database for familial melanoma.** *D.C-Y. Fung<sup>1</sup>, E.A. Holland<sup>1,4</sup>, B. Bressac-de Paillerets<sup>2,4</sup>, T.M. Becker<sup>1</sup>, N.K. Hayward<sup>3,4</sup>, G.J. Mann<sup>1,4</sup>.* 1) Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, NSW, Australia; 2) Institut Gustav Roussy, Villejuif, France; 3) Queensland Institute for Medical Research, Herston, QLD, Australia; 4) Melanoma Genetics Consortium.

A proportion of melanoma-prone individuals in both familial and non-familial contexts has been shown to carry inactivating mutations in either *CDKN2A* or, rarely, *CDK4*. *CDKN2A* is a complex locus that encodes two unrelated proteins from alternately spliced transcripts that are read in different frames. The alpha transcript (exons 1a, 2 and 3) produces the p16INK4A cyclin-dependent kinase inhibitor, while the beta transcript (exons 1b and 2) is translated as p14ARF, a positive regulator of p53 levels through binding to hDM2. Variants in exon 2 can affect both proteins and insertions and deletions in exons 1a, 1b and 2 can theoretically generate p16INK4A-p14ARF fusion proteins. No online database currently takes into account all the consequences of these genotypes, a situation compounded by some problematic previous annotations of *CDKN2A*-related sequences and descriptions of their mutations. We are therefore establishing a database of germline variants observed in all loci implicated in familial melanoma susceptibility. It collates information for each reported genotype on its molecular outcomes, historical description, methods of detection, biological effects and population genetics. The database was developed on the Linux/Intel platform and is managed under the DBMS, PostgreSQL 7.0. Java servlets contained within an Apache Tomcat-Jakarta 3.2 server are being developed for handling the data querying logic and posting HTML reports to a client-side browser. The database is being used to organise information about melanoma susceptibility gene variants for the Melanoma Genetics Consortium and will be made publicly available to facilitate research and clinical investigation of melanoma predisposition.

**Relationship of P gene with human eye color and dysplastic nevi or melanoma.** *T.R. Rebbeck<sup>1</sup>, P.A. Kanetsky<sup>1</sup>, A.H. Walker<sup>1</sup>, R. Holmes<sup>1</sup>, A.C. Halpern<sup>2</sup>, D.E. Elder<sup>1</sup>, D. Guerry<sup>1</sup>.* 1) University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Memorial Sloan-Kettering Cancer Center, New York, NY.

Multiple genes have been inferred to play a role in the determination of eye color, and eye color has been reported as a melanoma risk factor. The P gene, the human homologue to the mouse pink-eye dilution locus, is involved in the regulation of human pigmentation. Mutations in the P gene are associated with oculocutaneous albinism type 2 and other phenotypes that confer eye hypopigmentation. The P gene is located on chromosome 15q11.2-q12, which is also the location of a putative eye pigmentation gene (EYCL3) inferred to exist by linkage analysis. Therefore, the P gene is a strong candidate for determination of human eye color. We studied a sample of 681 individuals with invasive melanoma (MM) with or without dysplastic nevi (DN), DN only, and controls. Subjects were significantly less likely to have blue or gray eyes if they had P gene variants Arg305Trp ( $p=0.002$ ), Arg419Gln ( $p=0.001$ ), or the combination of both variants ( $p=0.003$ ). In individuals who carry Trp305 alleles, we also identified an increased risk of DN (OR=2.4, 95%CI: 1.2-4.8) but a protection from MM among individuals who had a previous diagnosis of DN (OR=0.4, 95%CI: 0.2-0.9). These relationships were strongest among those who carried both Trp305 alleles and who did not have blue or gray eyes (OR=3.4, 95%CI: 1.3-9.0 for DN vs. controls, and OR=0.2, 95%CI: 0.05-0.6 for DN+MM vs. DN only). These results suggest that P gene may in part determine human eye color, that Arg305Trp genotypes predict propensity to develop DN, and that these DN tend not to be associated with development of invasive melanoma. Although melanoma risk is increased overall in patients with DN, most such patients do not develop melanoma. These results suggest a possible means of prospectively identifying patients with DN who do not progress to develop melanoma.

**A conditional mouse knockout model of MEN1 develops pancreatic tumors when crossed with transgenic lines expressing Cre from the insulin promoter.** *J.S. Crabtree*<sup>1</sup>, *J.M. Ward*<sup>2</sup>, *L. Garrett-Beal*<sup>1</sup>, *P.C. Scacheri*<sup>1</sup>, *J. Hagar*<sup>3</sup>, *M. Inoue*<sup>3</sup>, *D. Hanahan*<sup>3</sup>, *H. Edlund*<sup>4</sup>, *S.C. Chandrasekharappa*<sup>1</sup>, *S.J. Marx*<sup>5</sup>, *A.M. Spiegel*<sup>5</sup>, *F.S. Collins*<sup>1</sup>. 1) NHGRI/NIH, Bethesda, MD; 2) Veterinary Pathology, NCI/NIH, Frederick, MD; 3) Dept of Biochemistry, UCSF, San Francisco, CA; 4) Dept of Microbiology, University of Umea, Umea, Sweden; 5) NIDDK/NIH, Bethesda, MD.

Multiple endocrine neoplasia, type 1 (MEN1) is an autosomal, dominant, familial cancer syndrome characterized primarily by multiple tumors in the parathyroid, endocrine pancreas and anterior pituitary. Other tumors, including gastrinoma, carcinoid, adrenal cortical tumors, angiofibroma, collagenoma and lipoma also occasionally occur. Affected families are almost invariably found to have germline loss of function mutations in the *MEN1* gene, and tumors are found to have lost the wild type allele.

To examine the mechanism by which loss of *MEN1* leads to tumor formation, we have generated a conditional mouse model through homologous recombination of the mouse homolog, *Men1*. A PGK-neomycin cassette flanked by loxP sites was inserted into intron 2 and a third loxP site was introduced into intron 8 of the *Men1* gene. These mice were then crossed with a ubiquitous cre expressing line, EIIa-Cre, to remove the PGK-neomycin cassette, generating *Men1<sup>dN</sup>* mice with a floxed allele.

*Men1<sup>dN</sup>* mice were crossed with two different transgenic lines expressing cre under the control of the rat insulin promoter (RIP-Cre). Mice that are positive for RIP-Cre develop pancreatic islet hyperplasia and frank adenoma, and there is a suggestion that the animals homozygous for the floxed allele have an earlier onset than the heterozygotes. Insulin levels in these mice increase with the onset of tumors, indicating beta cell specificity. In some cases, there is a marked decrease in blood glucose. Islet beta cell-specific knockout of the *Men1* gene appears to be an excellent animal model of insulinoma.

**Assessment of Protein Kinase A subunits expression in normal adrenal cortex and primary pigmented adrenocortical disease from patients with and without Carney Complex and *PRKARIA* mutations.** *F. Sandrini*<sup>1</sup>, *L.S. Kirschner*<sup>1</sup>, *A.J. Carney*<sup>2</sup>, *C.A. Stratakis*<sup>1</sup>. 1) UGEN, DEB, NICHD, NIH, Bethesda, MD; 2) Emeritus Staff, Mayo Clinic, Rochester, United States.

The primary pigmented nodular adrenocortical disease (PPNAD) is an endocrine tumor frequently associated to Carney Complex (CNC)- a familial multiple neoplasia syndrome. Forty percent of the families with CNC present mutation in the gene encoding the protein kinase A type I-alpha regulatory subunit (*PRKARIA*). We studied PPNAD from CNC patients with and without mutation at *PRKARIA* gene (mut or non-mut) and we compared them with a normal adrenals and ACTH induced adrenal hyperplasia. We quantitated the expression of the genes which encode the regulatory subunits (Ia, Ib, IIa, IIb) and the catalytic subunit alpha (Ca) by using a real-time reverse transcription (RT)-polymerase chain reaction (PCR) assay. We performed immunohistochemistry (IHC) with a monoclonal antibody specific for RIa, IIa, IIb and Ca (RIa- Ab, IIa- Ab, IIb-Ab and Ca- Ab ) in PPNAD and the adrenal hyperplasia. Microdissection was also done in PPNAD, and the gene expression of the tumoral cells was compared to those peritumoral cells. The *PRKARIA* gene is lower expressed in PPNADmut and hyperplastic adrenals than in PPNADnon-mut. In both of them, the *PRKARIA* is lower expressed when compared with normal adrenals. The *PRKAR2B* expression was higher expressed in both tumor cell lines than in that from normal adrenals. But the PPNADnon-mut had higher expression than the others cell lines. The *PRKACA* was lower expressed in both tumors than in normal adrenal, and the PPNADnon-mut was even lower than the others were. IHC showed low and irregular expression of the RIa subunits in the PPNADmut nodules, in contrast with other samples that showed high and regular expression. The same pattern is observed with Ca. The RIIa-Ab stained regularly the PPNADnon-mut and hyperplastic adrenals, but it irregularly stained the PPNADmut. The PPNADnon-mut expresses regularly the RIIb and it is irregularly expressed in hyperplastic adrenals and PPNADmut. There is a shift between RIa and IIb in the tumoral cells in adrenal tumors from CNC with *PRKARIA* mutate gene.

**Association between the MnSOD polymorphism (Ala-9 Val) and the adenomas of the distal colon.** *E.H. Elkhoully<sup>1</sup>, A.J. Levine<sup>1</sup>, A.T. Diep<sup>1</sup>, E.R. Lee<sup>2</sup>, H.D. Frankl<sup>3</sup>, R.W. Haile<sup>1</sup>.* 1) Preventive Medicine, USC Keck School of Medicine, Los Angeles, CA; 2) Division of Gastroenterology, Kaiser Permanente Medical Center, Bellflower, Los Angeles, CA; 3) Division of Gastroenterology, Kaiser Permanente Medical Center, Sunset, Los Angeles, CA.

Reactive oxygen species formed during normal metabolic processes can cause DNA damage and potentially increase cancer risk. MnSOD is an intramitochondrial isoenzyme of the superoxide dismutase family. The enzyme protects DNA against free radical induced DNA and protein damage by scavenging the free radicals. A common polymorphism in the mitochondrial targeting sequence, a Valine to Alanine substitution at position-9 (Shimoda-Matsubayashi, S., *Biochem. Biophys. Res. Commun.*, 266, 561-565) has been associated with increased premenopausal breast cancer risk and with colon cancer risk in individuals less than 40 years of age. Using an Alu I RFLP, we genotyped a random sample of 250 subjects (106 cases and 144 controls with a mean age of 69), from a sigmoidoscopy-based case-control study in Southern California. After adjusting for age, race, sex, cilinc and sigmoidoscopy date, we observed a borderline significant decrease in distal adenoma risk associated with the presence of at least one alanine allele: AA versus VV: OR =0.65(95% confidence interval (CI) 0.30-1.42), AV versus VV: OR=0.56(0.29-1.08), and AV+AA versus VV: OR=0.58 (0.31-1.10). If these findings are confirmed, it suggests that this MnSOD mutation might be protective against distal adenomas in older individuals.



**Methionine Synthase Reductase Polymorphism 66A-G and Sigmoid Colon Polyp.** *Y. Liu<sup>1</sup>, A.J. Levine<sup>1</sup>, A.T. Diep<sup>1</sup>, E.R. Lee<sup>2</sup>, H.D. Frankl<sup>3</sup>, R.W. Haile<sup>1</sup>.* 1) Preventive Medicine, USC Norris Cancer center, Los Angeles, CA; 2) Division of Gastroenterology, Kaiser Permanente Medical Center, Bellflower, Los Angeles CA; 3) Division of Gastroenterology, Kaiser Permanente Medical Center, Sunset, Los Angeles CA.

Methionine synthase transfers a methyl group from 5-methyltetrahydrofolate to homocysteine to form methionine in a vitamin B12 dependent reaction. The final product in this pathway is S-adenosylmethionine, the universal methyl donor for all intracellular transmethylation reactions. Methionine synthase reductase (MSR) maintains methionine synthase in a functional state for homocysteine to methionine remethylation. A mutation in MSR causes cbIE, a life-threatening genetic disorder in homocysteine methylation, documenting its functional importance in one-carbon metabolism. We assessed a common polymorphism in MSR (I66M) that has recently been associated with a 3-fold increase in the risk of a Down Syndrome as part of a larger study of risk factors for DNA methylation changes in colorectal adenoma. Since normal folate metabolism may be an important modifying factor in colorectal neoplasia risk, we tested the hypothesis that the MSR I66M polymorphism was associated with increased colorectal adenoma risk. Using a PASA method, we genotyped a random sample of 268 subjects (125 cases with distal adenomas and 143 controls), from a sigmoidoscopy-based case-control study in Southern California. 28 cases and 31 controls were homozygous for the methionine allele. Compared to homozygous wild types, those methionine homozygotes were not at greater risk for distal adenomas in this population (OR= 1.26; 95% Confidence Interval = 0.64 - 2.48). These data do not support an important role for the MSR protein in distal colorectal adenoma risk.

**The SOD2 Val 16 Ala polymorphism and Prostate Cancer.** *R.M. Sachdeva<sup>1</sup>, A-M. Martin<sup>2</sup>, M.F. Heyworth<sup>1,2</sup>, C.M. Zeigler-Johnson<sup>2</sup>, E. Spangler<sup>2</sup>, A.H. Walker<sup>2</sup>, S.B. Malkowicz<sup>2</sup>, T.R. Rebbeck<sup>2</sup>.* 1) VA Medical Center, Philadelphia, PA; 2) Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

SOD2 (Mn-SOD), an oxidoreductase, is a member of the superoxide dismutase group of the antioxidant superfamily of enzymes. The human SOD2 gene is on chromosome 6q25.3-qter and encodes a 24 amino acid mitochondrial target sequence. SOD2 inactivates anionic reactive oxygen species (ROS) that are by-products of oxidative phosphorylation. ROS are known to produce noxious effects within organic macromolecules including DNA and RNA strand scission, membrane lipid peroxidation and protein cross-linking. Homozygotes for the Ala allele of the Val 16 Ala polymorphism in SOD2 have been reported to be associated with an increase in breast cancer risk among Caucasian women. To evaluate the association of this SOD2 polymorphism with risk for prostate cancer, we studied 300 incident prostate cancer cases and 235 healthy male controls ascertained from the Hospital of the University of Pennsylvania between 1995 and 2000. The Val 16 Ala polymorphism was characterized by an allele-specific oligonucleotide PCR-RFLP assay with Cac 8I and confirmed with NgoM IV. Among controls, the Ala allele frequency was similar in Caucasians (Ala frequency=0.31) and African Americans (Ala frequency=0.25;  $c^2=1.7$ ,  $df=1$ ,  $p=0.19$ ). We identified a two-fold increase in prostate cancer risk in carriers of Ala/Ala genotypes compared with Val/Val or Val/Ala genotypes (age- and race-adjusted OR= 2.1, 95% CI: 1.5-3.1), which was similar in African Americans and Caucasians. We found no association with PSA level at diagnosis, or with tumor grade, presence of extracapsular extension, or other characteristics of tumor severity. These data suggest that SOD2 is a candidate gene for prostate cancer risk.

**Linkage of hereditary breast cancer families to a novel locus at 13q21-q22: subgroup analyses and further exclusion of BRCA2 involvement.** *S.H. Joo and Nordic/NHGRI Breast Cancer Consortium.* Columbia Univ Genome Center, NY, USA; NHGRI/NIH, Bethesda, MD, USA; Univ of Tampere, Helsinki, Finland; Univ Hospital, Lund, Sweden; Univ Hospital of Iceland, Iceland.

We recently reported a putative novel breast cancer susceptibility locus at 13q21-22 based on a targeted linkage analysis focusing at this common somatic deletion area in BRCA1/2-mutation negative hereditary breast cancers. The highest 2-point lod score in 77 families from Finland, Sweden and Iceland was 2.8 at D13S1308, 25 cM distal from BRCA2. This close proximity to the BRCA2 gene prompted us to further explore 13q21 linkage using a variety of strategies in these Nordic families. First, screening of BRCA1/2 for mutations in more affecteds led to the identification of an additional BRCA2 family. Furthermore, we also excluded another family with a significant lod score of 1.3 at BRCA2, but no detectable BRCA2 mutations. These changes and deletion of a previously unidentified MZ twin increased the lod score from 2.8 to 3.1 at D13S1308. Second, we typed additional markers along chromosome 13, including ~20 cM centromeric from BRCA2 at 13q11-q12. Small lod scores (~ 0.6) were found at 13q11-12, and no evidence of linkage at BRCA2. Third, we divided the families into those that met the Breast Cancer Linkage Consortium (BCLC) criteria and those that did not. The BCLC criteria define dense and early onset families. Evidence for 13q21-q22 linkage came mainly from families that did not meet BCLC criteria. These non-BCLC families had a lod score of 2.0 at D13S1308, and no evidence of linkage at 13q11-12. In conclusion, our data indicate that elevated lod scores at 13q21-q22 in Nordic BRCA1/2-mutation-negative breast cancer families are unlikely to be due to missed BRCA2 mutations. The phenotype of these families is also different from BRCA2 families.

**Tyrosine kinase activation in breast carcinoma with correlation to HER-2/neu gene amplification.** *R. Bhargava<sup>1</sup>, R. Naeem<sup>1</sup>, S. Marconi<sup>1</sup>, J. Luszcz<sup>1</sup>, J. Garb<sup>1</sup>, R. Gasparini<sup>2</sup>, C.N. Otis<sup>1</sup>.* 1) Department of Pathology, Baystate Medical Center, Springfield, MA; 2) Ventana Medical Systems.

**Purpose of Study:** The HER-2/neu oncogene encodes a transmembrane receptor with intrinsic tyrosine kinase activity. A pilot study was performed to investigate downstream effects of HER-2/neu (or related growth factor receptor) activation in breast carcinoma by identifying phosphorylated tyrosine. **Materials and Methods:** Fifty breast carcinomas were evaluated for HER-2/neu gene amplification by fluorescence in situ hybridization (FISH) using the Ventana gene probe. To exclude the possibility of tetraploidy in tumor cells, more than four signals per nucleus was considered as an evidence of gene amplification. Phosphotyrosine (an indication of tyrosine kinase activity) was detected by an antiphosphotyrosine (APT) mouse monoclonal antibody (Upstate Biotechnology). Intense APT immunoreactivity in tumor cells was considered as evidence of increased tyrosine kinase activity. **Results:** The HER-2/neu oncogene amplification was detected in 28% (14/50) cases. Increased tyrosine kinase activity was detected in 32% (16/50) cases. There was moderate agreement between HER-2/neu gene amplification and tyrosine kinase activity ( $k = 0.43$ ). Of the 14 cases demonstrating oncogene amplification, tyrosine kinase activity was detected in 9 (64.2%) cases. Of the 16 cases demonstrating increased tyrosine kinase activity, HER-2/neu oncogene amplification was identified in 9 (57%) cases. **Conclusion:** HER-2/neu oncogene amplification correlated to increased tyrosine kinase activity, supporting the mechanism of tyrosine kinase activation by HER-2/neu amplification. However, seven cases demonstrating increased tyrosine kinase activity did not show gene amplification raising the possibility of other growth factor receptors operating via tyrosine kinase pathway. The result of this pilot study suggest that a large scale investigation into downstream activation of tyrosine kinase and correlation to clinical outcome or response to Herceptin therapy may identify subsets of patients whose clinical response or outcome may be predicted by tyrosine kinase activation.

**Identification of regions of amplification on chromosome 19 in acute myeloid leukemia.** *S.N.J. Sait<sup>1</sup>, S. Kakati<sup>1</sup>, S. Matsui<sup>2</sup>, J.M. Conroy<sup>3</sup>, N.J. Nowak<sup>3</sup>, M.R. Baer<sup>4</sup>.* 1) Clinical Cytogenetics Lab, Roswell Park Cancer Institute, Buffalo, NY; 2) Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 3) Microarray & Genomics Facility, Roswell Park Cancer Institute, Buffalo, NY; 4) Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY.

Amplification of CMYC and MLL is well-documented in patients with acute myeloid leukemia, but reports of other gene amplifications are infrequent. Karyotypic analysis of pretreatment marrow cells from two patients with acute myeloid leukemia showed the presence of marker chromosomes with homogenously staining regions. The regions were identified to originate from chromosome 19 by spectral karyotyping and fluorescence in situ hybridization (FISH) using whole chromosome paints. BAC clones for chromosome 19 identified from the Human BAC Resource (<http://genomics.roswellpark.org>) were mapped by FISH to further define the regions of amplification. Amplification of a BAC that mapped to 19q12 and contained the markers D19S409, D19S882 and the gene for ubiquinol-cytochrome C reductase iron-sulfur subunit (UQCRC1) (<http://genome.cse.ucsc.edu>). was demonstrated in cells from one patient. Overlapping clones are being tested to map boundaries of the region involved as well as to identify what other regions on 19 are involved. Regions on chromosome 19 particularly cyclinE have been reported amplified in solid tumors. They may also play a role in hematologic malignancies.

**Altered subcellular distribution of OVCA1, a candidate tumor suppressor and its interactor, RBM8, a highly conserved RNA binding protein, in response to osmotic shock: implications for changes in mRNA processing in response to stress.** A.H. Prowse, A.M. Salicioni, A.K. Godwin. Fox Chase Cancer Ctr, Philadelphia, PA.

Allelic loss of chromosome 17p13.3 is one of the most frequently observed molecular alterations observed in ovarian, breast and other tumors. We have identified a gene, *OVCA1*, within a region of minimal allelic loss in ovarian tumors at 17p13.3 and we have shown that *OVCA1* is a strong candidate for a tumor suppressor involved in ovarian cancer (Bruening *et al.*, 1999). *OVCA1* codes for a highly conserved protein with no known function. To help determine the functions of *OVCA1*, we used a yeast-2-hybrid screen to identify *OVCA1*-associating proteins. One such protein, RBM8, was identified. The sequence of RBM8 indicates that it is a new member of an RNA-binding motif family which is highly conserved (Salicioni *et al.*, 2000). *OVCA1* exists in at least two forms: a 48kDa (p48<sup>OVCA1</sup>) and a 50kDa (p50<sup>OVCA1</sup>) protein. Immunofluorescence studies in HeLa cells using antibodies specific for p48<sup>OVCA1</sup> and p50<sup>OVCA1</sup> show that both localize throughout the cell, albeit with different patterns. Interestingly, osmotic shock induces p50<sup>OVCA1</sup> to accumulate around the nucleus, but has no dramatic effect on p48<sup>OVCA1</sup>. RBM8, which is localized predominantly in a punctate pattern in the nucleus in untreated HeLa cells, has a diffuse staining pattern throughout the nucleus and cytoplasm after osmotic shock. RBM8, also known as Y14, has been shown to be a shuttling protein, which binds preferentially to spliced mRNA in the nucleus upstream of exon-exon junctions and remains in this position in the cytoplasm (Kim *et al.*, 2001). RBM8/Y14 may therefore be involved in communicating to the cytoplasm the location of exon and intron boundaries and may play an important role in post-splicing events such as mRNA transport and nonsense mediated decay. Alterations in the cellular distribution of RBM8 may therefore have important consequences for mRNA processing. Studies are underway to further determine how these alterations in localization are mediated and the relevance of this response to how tumor cells adapt to environmental stress.

**Random Activation of Gene Expression to Identify Proteins Regulating Apoptosis.** *S. Rundlett*. Research, Athersys, Inc., Cleveland, OH.

Random Activation of Gene Expression to Identify Proteins Regulating Apoptosis Over-expression of several proteins has been shown to correlate with both increased tumorigenicity and decreased sensitivity to various apoptotic inducing agents in tumor cells. Identification of novel proteins whose over-expression promotes resistance to apoptotic inducing agents would aid in the development of therapeutic approaches to treat cancer. We have used Random Activation of Gene Expression (RAGE) technology to generate a protein expression library in the human fibrosarcoma cell line HT1080, and this library has been screened for cells (RAGE-clones) resistant to apoptosis-inducing agents. Approximately two million RAGE-clones were separated into 10 pools and screened for resistance to paclitaxol or to the combination of either cycloheximide/TNF-alpha or cycloheximide/activating FAS antibody. A number of apoptotic-resistant clones were obtained from each of these treatments, many of which were resistant to the other two apoptosis-inducing treatments. Because RAGE transcripts have 5'-sequence tags, identification of the activated genes can be readily accomplished via 3'-RACE. Experiments are currently in progress to clone RAGE-activated cDNAs from these clones and verify their ability to promote apoptotic resistance.

**Lack of association of sporadic colorectal cancer with the MTHFR C677T mutation, plasma level and daily intake of folic acid, B12 and other B group vitamins.** *L. García<sup>1</sup>, M.A. López<sup>1</sup>, J. Arteaga<sup>1</sup>, O. Barrales<sup>2</sup>, M. Candelaria<sup>2</sup>, D. Green<sup>2</sup>, E. León<sup>2</sup>, X. López K.<sup>2</sup>, O.M. Mutchinick<sup>1</sup>.* 1) Dept de Genética; 2) Dept Hemato-Oncología Inst Nac Ciencias Médicas y Nutrición Salvador Zubirán, México,D.F.

**Background:** Colorectal cancer (CRC) is the third most common cancer cause of death. CRC etiology has been related to both genetic and environmental factors. Recent publications suggest that homozygosis for the MTHFR C677T mutation has a protective effect in the occurrence of CRC, although some others reject this possibility. The very high prevalence of the mutation and the TT genotype in the Mexican population (Mutchinick et al, Mol Genet Metab, 1999) and the existing controversy, prompted us to do the present study. **Objective:** To determine the prevalence of the TT genotype in CRC patients and two comparison groups to analyze a possible association of sporadic CRC with the MTHFR genotype and plasma level and food intake of folic acid (FA) and other B vitamins. **Design:** matched case control study. **Participants:** 71 CRC patients (33 females and 38 males), 33 with sporadic breast cancer (BC) and 38 with sporadic prostate cancer (PC) matched by age and sex to those of CRC. **Methods:** identification of the C677T mutation by PCR and restriction enzyme analysis. FA and B12 plasma level by Elisa and FA, B2, B6 and B12 intake by a food frequency intake questionnaire.

**Results:** Against all odds, the frequency of TT homozygotes was higher in CRC (38.0%) than in PC (28.9%) and BC (18.2%) patients. Even though the prevalence of TT in CRC patients was twice that in BC, no statistical differences (SD) were observed. All groups were in H-W equilibrium. No SD that suggests a possible association or interaction between any genotype, plasma FA and B12 and daily intake of B2, B6 and B12 and CRC was observed.

**Conclusions:** So far, our results reject a protective effect for sporadic CRC of the TT MTHFR genotype, of folates intake and plasma level, and of the other B group vitamins studied.



**Mutational analysis of APC gene, immunoreactivity of p53, and E-Cadherin protein in gastric adenocarcinoma.**

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Gastric cancer is one of the most prevalent cancers in the world particularly in Asia and Middle East. Unlike colorectal cancer, genetic alterations during the tumorigenesis of gastric cancer is not well characterized. APC, p53 and E-cadherin are implemented in the gastric cancer tumorigenesis. APC, a tumor suppressor gene controls b-catenin as a proto-oncogene and is believed to play a major role in the progression of early dysplasia of gastric epithelium into adenoma and adenocarcinoma particularly in well differentiated gastric cancer. P53 mutations have also been observed in adenoma and adenocarcinoma of the same type of tumors. Expression of E-Cadherin, a cell adhesion molecule, is diminished in gastric adenocarcinoma resulting in metastasis of these tumors. Mutational analysis and expression of these genes were studied in early and advanced gastric adenocarcinoma to elucidate their role in tumorigenesis of gastric cancer. Paraffin-embedded gastric adenocarcinoma including the normal margins from 22 patients, with endoscopy and/or gastrectomy (age group 32-70yrs) were screened for mutations in APC gene. APC coding regions, including 4 Mutation Cluster Regions (MCRs) in Exon 15 (1267-1588 cds) that are the hot-spots for nonsense mutations were examined by PCR-SSCP followed by sequencing of the suspected SSCP bands. 2/22 of adenocarcinomas had a mutation. These were new mutations in MCR-D (1503-1588 cds). Expression of mutant p53 and E-cadherin protein were measured by immunohistochemistry. 15/19 (79%) of patients showed mutated p53 and 9/19(47%) were negative for expression of E-cadherin. These data correlates with the reported results. A larger study on the genetic alterations of gastric cancer is underway. These data may demonstrate that the above genetic markers could be used as early diagnostic, &/or prognostic, predisposition factors for gastric cancer.

**Microsatellite instability versus immunohistochemistry testing in phenotyping colorectal tumors.** *N.M. Lindor<sup>1</sup>, L.J. Burgart<sup>1</sup>, O. Leontovich<sup>1</sup>, R.M. Goldberg<sup>1</sup>, J.M. Cunningham<sup>1</sup>, C. Walsh-Vockley<sup>1</sup>, G. Petersen<sup>1</sup>, M. Redston<sup>6</sup>, J. Young<sup>3</sup>, M. Barker<sup>3</sup>, M.D. Walsh<sup>2</sup>, J. Jass<sup>2</sup>, B.A. Leggett<sup>3</sup>, J. Hopper<sup>4</sup>, B. Bapat<sup>5</sup>, S. Gallinger<sup>5</sup>, T. Selander<sup>5</sup>, S.N. Thibodeau<sup>1</sup>.* 1) Mayo Clinic, Rochester, MN.,USA; 2) University of Queensland, Australia; 3) Bancroft Centre, Herston, QLD,3 Australia; 4) Univ. of Melbourne, Victoria, Australia; 5) Mount Sinai Hospital, Toronto, Ontario, Canada; 6) Harvard Medical School, Boston, Mass., USA.

**Purpose:** To compare microsatellite instability (MSI) testing with immunohistochemical (IHC) detection of hMLH1 and hMSH2 in colorectal cancer. **Patients and Methods:** Colorectal tumors from 1,144 patients from four cohorts from three countries were assessed for DNA mismatch repair deficiency by two methods: microsatellite instability testing and immunohistochemical detection of hMLH2 and hMSH2 gene products. High frequency microsatellite instability (MSI-H) was defined as >30% instability of at least 5 microsatellite markers. **Results:** Of 1,144 colorectal tumors tested for MSI and IHC, 818 showed intact expression of hMLH1 and hMSH2 by IHC. Of these, 680 were microsatellite stable (MSS), 27 were MSI-H, and 111 were MSI-L (1-29% of loci unstable). 228 tumors showed absence of hMLH1 expression and 100% of these were MSI-H. 98 tumors showed absence of hMSH2 expression and all but three showed unequivocal MSI-H. Discordance between MSI and IHC was partially explained by loss of expression of hMSH6, hPMS2, and, in the three discordant hMSH2 tumors, there were insufficient tumor cells to reliably interpret the MSI assay, however these patients had known germline hMSH2 mutations. **Conclusion:** IHC in colorectal tumors for protein products hMLH1 and hMSH2 provides a rapid, cost-effective, sensitive (92.3%), and extremely specific (100%) method for screening for DNA mismatch repair defects. Testing strategies must take into account acceptability of missing some cases of MSI-H tumors if only IHC is performed. Supported by NIH and UO1-CA74800, CA74778, and CA74783 (Cooperative Family Registry for Colon Cancer Studies)and CA68535. **Purpose***hMLH1hMSH2***Patients and Methods:**≥**Results****Conclusion****Purpose.**

**Transcriptional profiling of colon carcinomas, adenomatous polyps and normal colon mucosa using 36K**

**Microarrays.** *D. Hahnloser<sup>1</sup>, A. Berger<sup>3</sup>, T. Guillemette<sup>3</sup>, E. Christensen<sup>1</sup>, L. Burgart<sup>1</sup>, J.M. Cunningham<sup>1</sup>, L. Boardman<sup>1</sup>, S. Iturria<sup>2</sup>, B. Bryant<sup>3</sup>, M. Morrissey<sup>3</sup>, R. Schlegel<sup>3</sup>, S.N. Thibodeau<sup>1</sup>.* 1) Experimental Pathology, Mayo Clinic, Rochester, MN; 2) Health Sciences Research, Mayo Clinic, Rochester, MN; 3) Millennium Predictive Medicine, Cambridge, MA.

Genome-wide monitoring of gene expression can be used to identify sets of genes involved in carcinogenesis and may help to develop a molecular approach to classify tumors. Transcript expression profiles were analyzed from 25 colon adenocarcinomas, 3 adenomatous polyps and paired normal tissues. The tumors differed in location in the colon (proximal vs. distal), in microsatellite instability status (MSI vs. MSS), in stage of disease (Dukes A,B vs. Dukes C,D) and in the patients age at onset (50 vs. >50 years). Total RNA was extracted from normal, polyp and tumor tissues and radioactive probes were hybridized to nylon arrays containing 36,000 clones, including known genes and ESTs.

Expression levels were quantitated and analyzed with Array Vision, Cluster, TreeView, and additional software developed at Millennium Pharmaceuticals. Hierarchical clustering, using 3077 clones displaying the greatest differential expression (8.8% of clones on the arrays), correctly classified all samples as tumor, polyp or normal. These findings suggest that there are systematic differences between these three types of samples. The different subgroups of colon cancers could not be as closely related to a specific pattern of expression as were the normal, polyp or tumor tissues, but up and down regulated clones, clearly different between the subgroups, were identified. This analysis might be helpful in generating a molecular classification of cancers or in discovering previously unknown and clinically important subsets of tumors. The results from this large-scale expression analysis also serve to identify possible screening and prognostic markers for colorectal cancer and can give directions for future research.

**Evidence of different repertoires of MTG (ETO/CDR) oncoproteins in human myeloid leukemia.** *N. Sacchi*<sup>1,2</sup>, *J. Schonkeren*<sup>3</sup>, *A. Fenaroli*<sup>4</sup>, *L. Schiaffonati*<sup>4</sup>, *V. Stoyanova*<sup>3</sup>, *S. Rossetti*<sup>2,3</sup>, *L. van Unen*<sup>3</sup>, *A.T. Hoogeveen*<sup>3</sup>. 1) Oncology, Johns Hopkins University, Baltimore, MD; 2) School of Medicine, University of Milan, Italy; 3) Erasmus University, Rotterdam, The Netherlands; 4) Department of Biomedical Sciences and Biotechnology, University of Brescia, Italy.

The MTG8 (ETO/CDR), MTGR1 and MTG16 oncoproteins with a very high degree of sequence similarity with the *Drosophila* neryy are a novel family of transcriptional repressor molecules implicated in human leukemia and neurodegenerative disorders. Two of these proteins, MTG8 and MTG16, are found fused to AML1, one of the subunits of the hematopoietic core binding factor (CBF), as a consequence of the t(8;21) and t(16;21) associated with acute myeloid leukemia (AML) subtypes. We found that the intranuclear localization of the wild-type MTG16 protein differs from the localization of the other two MTG proteins, MTG8 and MTGR1. MTG16 is often localized both in the nucleoplasm and/or the nucleolus whereas MTG8 and MTGR1 are localized exclusively in the nucleoplasm. In contrast, the chimeric AML-MTG16, like AML1-MTG8, is localized in the nucleoplasm. Interestingly, all the MTG proteins can interact with each other and their interaction can influence their intranuclear localization. Both wild-type and chimeric MTG proteins can form homo- and hetero-dimers. Oligomerization seems to be crucial to ensure the recruitment of additional nuclear co-repressors complexes with histone deacetylase (HDAC) activity and enhance repression of transcription of target genes. The expression profile of the MTG isoforms is distinct in myeloid leukemia subtypes suggesting that the differential expression of wild-type and chimeric MTG proteins may determine not only the repertoire of possible MTG oligomers in a given leukemia, but also their intranuclear localization, their regulatory function and, ultimately, their leukemogenic properties. Acknowledgements: This work has been partially supported by an AIRC (Italy) grant to N.S.; SR is supported by a Foreign Exchange Fellowship, University of Milan, Italy.

**Expression Analysis of Chromosome 20 Genes in Prostate Cancers.** *L. Wang<sup>1</sup>, J. Monahan<sup>4</sup>, J. Cheville<sup>1</sup>, D.A. Elkins<sup>1,2</sup>, A. Wonsey<sup>4</sup>, J.M. Cunningham<sup>1</sup>, D.J. Schaid<sup>3</sup>, R. Schlegel<sup>4</sup>, S.N. Thibodeau<sup>1</sup>.* 1) Lab Medicine & Pathology, Mayo Clinic, Rochester, MN; 2) Urology, Mayo Clinic, Rochester, MN; 3) Health Sciences Research, Mayo Clinic, Rochester, MN; 4) Millennium Predictive Medicine, Cambridge, MA.

Prostate cancer (PC) is one of the most common human cancers, occurring in as many as 15% of men in the United States. Recent studies suggest that hereditary PC is a complex disease, involving multiple susceptibility genes and variable phenotypic expression. While conducting a genome-wide search on 162 North American families with > 3 members affected with PC, we found evidence for linkage to chromosome 20q13. This region is also known to be amplified in other tumor systems, particularly in breast cancer. As one of our efforts to clone a PC susceptibility gene (HPC20) localized to this region, we have used microarray technology to examine the expression levels of various genes localized to chromosome 20, and in particular, to 20q13. A total of 31 micro-dissected prostate cancer samples with various clinical and pathologic features and 25 normal prostate tissues were analyzed. Total RNA was extracted from normal and tumor tissue and radiolabeled probes were hybridized to a custom nylon array containing approximately 6000 clones, of which 270 were known genes and ESTs mapping to chromosome 20. Among the 270 genes/ESTs analyzed, only a few were found to have a median expression level at least 2 fold higher in the tumors when compared with the controls, and none were found to be down regulated by two fold. Increasing the number of markers on chromosome 20 and using more sophisticated algorithms for assessing chromosome location-specific expression patterns may provide the additional power needed to identify HPC20 in the 20q13 region and to test for possible regions amplification or deletion.

**Gene Expression Profiling of Clear Cell Renal Cell Carcinoma: Gene Identification and Prognostic**

**Classification.** *M. Takahashi*<sup>1</sup>, *D. Rhodes*<sup>2</sup>, *K. Furge*<sup>3</sup>, *H. Kanayama*<sup>4</sup>, *S. Kagawa*<sup>4</sup>, *B. Haab*<sup>2</sup>, *B.T. Teh*<sup>1</sup>. 1) Cancer Genetics, Van Andel Research Institute, Grand Rapids, MI; 2) DNA and Protein Microarray Technology, Van Andel Research Institute, Grand Rapids, MI; 3) Molecular Oncology, Van Andel Research Institute, Grand Rapids, MI; 4) Department of Urology, School of Medicine, University of Tokushima, Tokushima, Japan.

To better understand the molecular mechanisms that underlie the tumorigenesis and progression of clear cell renal cell carcinoma (ccRCC), we studied the gene expression profiles of 29 ccRCC tumors obtained from patients with diverse clinical outcomes using 21,632-cDNA microarrays. We identified gene expression alterations both that were common to most of the ccRCC studied and that were unique to clinical subsets. There was a significant distinction in gene expression profile between patients with a relatively non-aggressive form of the disease (100% survival after 5 years with the majority (15/17 or 88%) having no clinical evidence of metastasis) versus patients with a relatively aggressive form of the disease (average survival time 25.4 months with a 0% 5 year survival rate). Approximately 40 genes most accurately make this distinction, some of which have previously been implicated in tumorigenesis and metastasis. To test the robustness and potential clinical usefulness of this molecular distinction, we simulated its use as a prognostic tool in the clinical setting. In 96% of the ccRCC cases tested, the prediction was compatible with the clinical outcome, exceeding the accuracy of prediction by staging. These results suggest that two molecularly distinct forms of ccRCC may exist and that integration of expression profile data with other clinical parameters could serve to enhance the diagnosis and prognosis of ccRCC. Moreover, the identified genes provide insight into the molecular mechanisms of aggressive ccRCC and suggest intervention strategies.

**Genomic and chromosomal instability in endometrial cancer.** *S.P. Siah<sup>1, 2</sup>, S.L. Bawden<sup>1</sup>, G.D. Bennett<sup>1</sup>, G. Casey<sup>1</sup>, M. Davy<sup>3</sup>, T.J. Dodd<sup>4</sup>, R.L.P. Flower<sup>2, 5</sup>, A.J. Gordon<sup>2</sup>, D.M. Quinn<sup>2</sup>, A. Ruzkiewicz<sup>4</sup>, G.K. Suthers<sup>6</sup>, Z. Rudzki<sup>1</sup>.* 1) Div. Molecular Pathology, IMVS, Adelaide, SA, Australia; 2) School of Pharmaceutical, Molecular and Biomedical Sciences, University of South Australia, Adelaide, SA, Australia; 3) Gynaecological-Oncology Unit, RAH, Adelaide, SA, Australia; 4) Div. Tissue Pathology, IMVS, Adelaide, SA, Australia; 5) Dept. Transfusion, PaLMS, RNSH, St Leonards, NSW, Australia; 6) Familial Cancer Unit, SA Familial Cancer Service, WCH, North Adelaide, SA Australia.

Microsatellite instability (MSI), a marker of genomic instability, is detected in up to 34% of unselected endometrial cancers. It has been hypothesised that chromosomal instability, indicated by chromosomal abnormalities, is involved in the pathogenesis of the remaining cases.

We studied 48 formalin-fixed paraffin embedded endometrial cancers for evidence of MSI and aneuploidy. Samples were classified as MSI positive when 2 or more of the 10 loci used showed extra alleles in the tumour tissue DNA compared to normal tissue DNA. DNA analysis by flow cytometry was used to detect aneuploidy and tumours were classified as diploid, near diploid, tetraploid or aneuploid.

MSI was detected in 13 (27.1%) of 48 cases, of which ten cases (79.6%) were diploid or near diploid. Aneuploidy and tetraploidy were detected in 2 and 1 MSI positive cases respectively. Of the MSI negative cases (n= 35), a total of 18 cases (51.4%) were diploid and near diploid. Aneuploidy and tetraploidy were detected in 15 and 2 MSI negative cases respectively.

In this study, MSI positive endometrial cancers were found to be associated with diploid or near diploid DNA status. An association between aneuploidy and MSI negative endometrial cancers was also detected. Our results further showed that CIN is more frequently detected in endometrial cancers than in MSI positive cancers. This suggests that, like genomic instability, chromosomal instability plays a valid role in endometrial tumourigenesis. A significant proportion of cases do not appear to involve either mechanisms, suggesting that other mechanisms may be at work.

**Unmasking the Masked Mutation in Large Genes: Evaluation of Haploid Conversion Technology.** *A. Ganguly, C. George, G. Tan, J. Pain, T. Ganguly.* Dept of Genetics, University of Pennsylvania, Philadelphia, PA.

It has been suggested that a significant fraction of mutation negative individuals with genetic diseases including breast and ovarian cancer or colon cancer actually harbor mutations in cancer susceptibility genes. However the mutations are masked by the presence of the normal allele. In order to unmask the hidden mutations, we have used a commercially available mouse-human somatic cell hybrid generation facility (GMP Genetics, MA) whereby the two different alleles of specific chromosomes are separated and individually assayed for expression/mutation. We have used the hybrid cells for analyzing female hemophiliacs, individuals with clinical FAP, HNPCC and breast cancer. The genetic analysis on individual alleles require careful selection of primers for PCR amplification to exclude cross hybridization with the mouse genomic DNA. This method has allowed us to rule out genomic deletions in any of the genes investigated and further work is in progress to define possible rearrangements and/or regulatory mutations. The DNA supplied by the company is adequate for PCR amplification of regular sized PCR products. But complete analysis of large exon bearing genes like Factor VIII, BRCA1/2, APC and others require expanding the cells to a culture of larger volume in order to isolate appropriate quantities of DNA. Therefore, this method is useful as a research tool. But it requires a much larger sequencing effort followed by additional molecular analysis and is therefore intensive in terms of labor and expenses. Thus far we have had success in uncovering mutations in Factor VIII gene in female hemophiliacs while work is in progress on the other genes. The completed results of multiple gene analyses will be presented.



**Identification of large genomic deletions in *BRCA1* from patients with hereditary cancer risk using SNP haplotype pair analysis derived from clinical sequence data.** *B.C. Hendrickson, D. Pruss, A.M. Deffenbaugh, D.R. Nieslen, C.A. Gaglio, T. Scholl.* Myriad Genetic Laboratories, Inc., Salt Lake City, UT.

Five novel large deletions within *BRCA1* that remove exons were detected in anonymized samples originating from patients with hereditary risk for breast/ovarian cancer. Specimens were selected for a strong family history of breast and/or ovarian cancer and a negative clinical test for mutations in *BRCA1* and *BRCA2*. SNP haplotype-pair analysis was performed on 1,000 samples meeting these criteria using DNA sequence data obtained from clinical testing. A group of fourteen samples with haplotypes suggestive of large genetic rearrangements in *BRCA1* exon 16 were assayed for genomic deletions by PCR. Seven of these samples carried various large deletions. Breakpoints for these deletions resulted from Alu-mediated recombination. Four different deletions of both exons 16 and 17 were characterized. One deletion removed a region of 6.4 kb and occurred in three unrelated patients who identified their ancestry as Caribbean/Latin American. Studies are ongoing to determine the population prevalence of this mutation. Also, a 5.6 kb deletion of exons 15 and 16 was identified in one sample. The presence of these mutations was assessed in 420 anonymized samples from patients with breast/ovarian cancer, at least two relatives with cancer, and a negative clinical test result. No additional occurrence of any of the mutations was detected in this specimen set.

These results demonstrate that clinical SNP data is useful to identify genetic mutations undetectable by conventional PCR-based clinical approaches. The method is attractive since the analysis can be applied to any gene for which sufficient SNP haplotype data is available. These results support hypotheses presented elsewhere that genomic deletions in the *BRCA* genes are diverse. An automated method to incorporate large rearrangement detection is currently under development and will be included with the highly sensitive full sequence commercial analysis available for the *BRCA* genes.

**Spinal tumor genotype-phenotype correlation in neurofibromatosis 2.** *M.E. Baser*<sup>1</sup>, *D.M. Parry*<sup>2</sup>, *J.M. Friedman*<sup>3</sup>, *A. Wallace*<sup>4</sup>, *J. Gillespie*<sup>5</sup>, *D.G.R. Evans*<sup>4</sup>. 1) Los Angeles, CA, U.S.A; 2) Genetic Epidemiology Branch, National Cancer Institute, Bethesda, MD, U.S.A; 3) Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada; 4) Department of Medical Genetics, St. Mary's Hospital, Manchester, U.K; 5) Department of Radiology, Manchester Royal Infirmary, Manchester, U.K.

Constitutional nonsense and frameshift mutations in the neurofibromatosis 2 (*NF2*) tumor suppressor gene are associated with severe NF2, one characteristic of which is a higher number of spinal tumors. Patronas et al. (Radiology 2001;218:434-442) reported that among 37 NF2 patients, those with nonsense or frameshift constitutional *NF2* mutations had more spinal intradural extramedullary tumors, but not spinal intramedullary tumors, than those with other types of mutations. We re-examined this question in patients from the United Kingdom NF2 registry. Of 86 U.K. patients with spinal MRIs, 54 had identified *NF2* mutations; excluding seven known somatic mosaics, the study group consisted of 47 patients (14 large deletions, 12 nonsense mutations, 11 splice-site mutations, nine missense mutations, one in-frame deletion) from 30 families. Intradural extramedullary tumors were more common in patients with nonsense mutations (100%) than in those with missense mutations (33%;  $P = .006$ ), splice-site mutations (54%;  $P = .014$ ), or large deletions (64%;  $P = .042$ ). Among NF2 patients with one or more intradural extramedullary tumors, individuals with nonsense mutations had significantly more tumors (mean  $\pm$  SE,  $16.4 \pm 5.0$ ) than those with missense mutations ( $3.6 \pm 1.5$ ;  $P = .041$ ) or large deletions ( $4.8 \pm 2.0$ ;  $P = .050$ ) (splice-site mutations,  $5.2 \pm 2.8$ ;  $P = .234$ ). In contrast, the prevalence and number of intramedullary tumors did not vary significantly by type of constitutional *NF2* mutation. These results are consistent with those of Patronas et al., and studies in which other tumors (intracranial meningiomas and peripheral nerve tumors) were found to be more common in NF2 patients with nonsense or frameshift *NF2* mutations.

**Investigation of interaction between N-acetyltransferase 2 (NAT2) phenotype and heterocyclic amines as potential risk factors for colorectal cancer.** *J.H. Barrett<sup>1</sup>, D.T. Bishop<sup>1</sup>, R. Waxman<sup>2</sup>, D. Forman<sup>2</sup>, T. Lightfoot<sup>3</sup>, C. Garner<sup>3</sup>, G. Smith<sup>4</sup>, R. Wolf<sup>4</sup> and FSA Study Group.* 1) Imperial Cancer Research Fund Genetic Epidemiology Division, Leeds, UK; 2) University of Leeds, UK; 3) University of York, UK; 4) University of Dundee, UK.

Individuals who are fast NAT2 acetylators may be at increased risk of colorectal cancer through the activation of carcinogenic heterocyclic amines, which are produced by meat cooked at high temperatures and are found in cigarette smoke. To investigate this hypothesis a study of 500 incident colorectal cancer cases and population controls, matched for age, sex and general practitioner, was conducted in the United Kingdom. Usual meat intake and lifetime smoking habits were estimated using a detailed food frequency questionnaire administered by interview. Subjects indicated how well cooked they ate their meat by responding to verbal or picture prompts. Subjects were classified as fast or slow NAT2 acetylators on the basis of NAT2 genotype using previously described phenotype-genotype correlations. Complete data were available on 433 matched pairs. The risk of colorectal cancer showed a steady increase with meat intake, rising to an odds ratio of 1.68 (95% confidence interval (1.11, 2.54)) for the highest versus the lowest quartile, and this was even more pronounced for red meat (odds ratio 2.33 (1.56, 3.48)). However this effect was not influenced by the preference for well-done meat. Smoking was also associated with an increased risk (odds ratio 1.47 (1.10, 1.98) for ever- versus never-smokers). In both cases and controls approximately 40% of subjects were classified as fast acetylators, and the risks associated with (red) meat intake and smoking did not vary according to NAT2 phenotype. This study provides no support for the hypothesis that fast NAT2 acetylators are at increased risk of colorectal cancer, even if exposed to high levels of heterocyclic amines from well-cooked meat or smoking. The effect of meat, which has been observed previously, could be due to fat content or to other correlates of high meat consumption in diet or lifestyle.

**DNA polymorphisms in XRCC1, XRCC2, XRCC3, ERCC4, PCNA genes and DNA-adduct levels in healthy subjects.** *G. Matullo*<sup>1,2</sup>, *S. Polidoro*<sup>3</sup>, *S. Guarrera*<sup>1,3</sup>, *M. Peluso*<sup>4</sup>, *A. Munnia*<sup>4</sup>, *A. Piazza*<sup>1</sup>, *D. Palli*<sup>5</sup>, *P. Vineis*<sup>3</sup>. 1) Dip. Genetica, Biol. e Biochim, Univ. Torino, Italy; 2) ISI Foundation, Torino; 3) Dipartimento di Scienze Biomediche e Oncologia Umana, Univ. Torino; 4) Unit of Experimental Oncology, IST, Genova; 5) Epidemiology Unit, CSPO, Firenze, Italy.

Polymorphisms in several DNA repair genes have been identified and individuals with non-dramatic reductions in the capacity to repair DNA damage are observed in the population. We investigated in 218 Italian healthy individuals belonging to the prospective European project EPIC the relationship between DNA damage, as measured by bulky DNA adduct levels, and the following genetic polymorphisms: XRCC1-26304C/T, XRCC1-26651A/G, XRCC2-31479G/A, XRCC3-17993A/G, ERCC4-30147A/G, PCNA-6084G/C. DNA-adduct levels were measured as Relative Adduct Level (RAL) per  $10^9$  by 32P DNA postlabeling assay in white blood cells from peripheral blood. Genotyping was performed by PCR-RFLP and/or DHPLC techniques. XRCC3-17993 AA genotype was significantly associated ( $P = 0.018$ ; OR = 2.08, 95%CI, 1.16-3.74) with higher mean DNA-adduct levels ( $10.48 \pm 1.34$ ) compared to AG+GG genotypes ( $6.64 \pm 0.88$ ). The PCNA-6084 GG genotype seems also to be associated ( $P = 0.042$ ) with higher DNA adduct levels ( $9.95 \pm 1.15$ ) compared to CG+CC genotypes ( $6.54 \pm 1.19$ ). In order to identify polymorphisms possibly related to the highest DNA adduct levels we defined a cut-off value above and below the 80th percentile (RAL = 13). The genotype distribution is still significantly different for XRCC3-17993A/G polymorphism (AA vs AG+GG,  $P = 0.026$ ), whereas it is not for PCNA-6084G/C polymorphism. Moreover, a borderline significance in genotype distribution has been observed also for XRCC2-31479G/A polymorphism (GG vs GA+ AA,  $P = 0.047$ ) and XRCC1-26651A/G (GG vs GA+ AA,  $P = 0.059$ ). Our results support the possible XRCC3 involvement in DNA bulky adduct repair, as we already suggested for another XRCC3 polymorphism (Thr241Met, exon 7), indicating that this gene can possibly repair bulky adducts through alternative repair pathways or repairing DNA cross-link lesions detected by 32-P DNA postlabelling technique.

**Genotypic and Phenotypic Characterization of a Putative Tumor Suppressor Gene *GNMT* in Liver Cancer.** T.-L.

Tseng<sup>1</sup>, Y.-M. Chen<sup>2</sup>, Y.-P. Shin<sup>2</sup>, K.H. Buetow<sup>1</sup>. 1) Lab of Population Genetics, NCI, Bethesda, USA; 2) Institute of Public Health, National Yang-Ming University, Taiwan.

Glycine N-methyltransferase (GNMT) is a protein with multiple functions related to maintain the genetic stability of cells, and it has been shown to be down-regulated in human HCC. Here we are investigating the *GNMT* gene for liver cancer predisposition by genotypic and phenotypic characterization of *GNMT* in Chinese population and hepatoma cell lines. Two STRPs, one Insertion/Deletion and three SNPs were identified and confirmed. We further developed genotyping assays and quantitative methods for assessing allelic loss at the *GNMT* locus. The genotypic distribution of *GNMT* between normal and HCC PBL DNA was similar; however, it was very different between PBL DNA and the tissue DNA from HCC patients. The rates of heterozygosity of STRP1, SNP1, SNP2, and STRP2 decreased dramatically in both non-tumor and tumor liver tissues DNA from HCC patients compared with the PBL DNA. This may result from the high LOH rate within the *GNMT* gene in liver. To elucidate the functional impact of polymorphisms in the promoter region, we performed luciferase reporter gene and gel mobility shift assays with hepatoma cell lines. The reporter gene assays indicate that the transcriptional activity of the *GNMT* promoter is strongly associated with the length of GA repeats in STRP1. The transcriptional activity of the *GNMT* promoter containing long repeats was reduced to 40~60% of the promoter containing short repeats. The gel mobility assays indicate that the short allele of Ins/Del polymorphism abolishes an HNF-3 recognition site in the *GNMT* promoter. Therefore, the two polymorphisms in the promoter region could be functional polymorphisms. Based upon the phenotypic results, we selected the risk genotypes and estimated their contribution to the genetic susceptibility to liver cancer. The average of odds ratio for the risk genotypes of *GNMT* in liver tissue, as compared with non-risk genotypes, is about 1.37~3.97. Finally, the somatic instability of *GNMT* in the early event of HCC development and the diminution of *GNMT* expression in HCCs suggest that *GNMT* could be a putative tumor suppressor gene in liver cancer.

**Gene Expression Profiling of Breast Cancer Biopsies with Cellular Resolution.** *M. Erlander<sup>1</sup>, X. Ma<sup>1</sup>, R. Salunga<sup>1</sup>, T. Tuggle<sup>1</sup>, E. Enright<sup>1</sup>, T. Payette<sup>1</sup>, Y. Tran<sup>1</sup>, P. McQuarry<sup>1</sup>, M. Pistone<sup>1</sup>, K. Stecker<sup>1</sup>, D. Sgroi<sup>2</sup>, J. Wittliff<sup>3</sup>.* 1) Arcturus Applied Genomics, Calrsbad, CA; 2) Harvard Medical School Boston, MA; 3) University of Louisville, Louisville, KY.

Breast cancer biopsies contain a multitude of cell types in addition to supposed pre-cancerous cells (ductal carcinoma) and carcinoma (invasive ductal carcinoma). Because of the heterogeneous nature of breast biopsies, interpretation of gene expression profiling of whole biopsies is problematic and requires a number of assumptions in the subsequent bioinformatic analyses. In addition, subsequent validation of differential gene expression requires the determination of which cell type(s) is expressing the gene(s) of interest. To obviate these shortcomings, we have implemented a general approach of laser-capturing cells from 5 tissue sections followed global amplification of mRNA and subsequent gene expression profiling via microarrays. Currently two projects are ongoing with the most recent data from both presented. First, the progression of breast cancer is being studied by examining from individual breast biopsies the gene expression (2500 genes profiled) of captured cells from normal epithelium, DCIS and IDC. High correlations ( $r > 0.95$ ) were observed between three individual captures and amplifications (106-fold amplifications) for all three cell types. In patients profiled so far, normal, DCIS and IDC have unique gene expression patterns and this data will be presented. The second project is a retrospective gene expression profiling of breast cancer biopsies taken on average 8 years ago. In this project gene expression analysis of laser-captured IDC is being linked to pathological, biochemical, and clinical datasets to define via gene expression patterns (i.e., signatures) the molecular subtypes of breast cancer. In patients studied so far, high correlation ( $r > 0.95$ ) of gene expression patterns has been observed between captures/RNA amplifications and gene expression patterns are consistent with known biomarker data. Both projects should lead to a greater understanding of breast cancer as well as uncover novel diagnostic and prognostic markers/signatures and new therapeutic targets.

**Identifying genes involved in genetic predisposition to papillary thyroid carcinoma.** *Y. Huang<sup>1</sup>, N. Pellegata<sup>1</sup>, W. Frankel<sup>2</sup>, V. LiVolsi<sup>4</sup>, C. Eng<sup>1,3</sup>, H. Hampel<sup>3</sup>, W. Lemon<sup>1</sup>, F. Wright<sup>1</sup>, K. Kornacker<sup>5</sup>, R.T. Kloos<sup>6</sup>, W.B. Farrar<sup>7</sup>, D.E. Schuller<sup>8</sup>, R. Krahe<sup>1</sup>, A. de la Chapelle<sup>1</sup>.* 1) Human Cancer Genetics, Ohio State U, Columbus, OH; 2) Dept Pathol, OSU; 3) Clinical Cancer Genetics, OSU; 4) Dept Pathol & Lab Med, U Penn, Philadelphia, PA; 5) Dept Sensory Biophys, OSU; 6) Div Endocrinol & Nucl Med, OSU; 7) Dept Surg, OSU; 8) Dept Otolaryngol, OSU, OH.

Thyroid cancer is the most common endocrine malignancy and can occur as either familial or sporadic disorder. Papillary thyroid carcinoma (PTC), the most common type, accounts for 65-80% of all thyroid cancers. Familial PTC accounts for about 6% of all PTCs. We hypothesize that there are genes that when mutated lead to inherited predisposition to PTC. To identify such putative genes we postulated that they might be commonly down-regulated in the PTCs. We used oligo-based DNA microarrays (U95A, Affymetrix) to analyze eight PTCs in comparison with matched normal tissue from the same individual. One of the eight patients has a family history of PTC. Preliminary analyses suggest that the study design was successful. 1) Genes encoding enzymes involved in reactions responsible for the production of thyroid hormone, e.g. thyroid peroxidase, type I and type II iodothyronine deiodinase, were down-regulated in the majority of tumors, as might be expected when comparing cancer tissue with normal thyroid tissue. 2) Genes previously found over-expressed in PTC, such as MET, and those encoding galectin-3, fibronectin, a1-antitrypsin, keratin 19 and liver dipeptidyl peptidase IV, were up-regulated in all eight PTCs. The RET proto-oncogene was expressed in four PTCs. 3) We have confirmed the up-or down-regulation of selected genes by semi-quantitative RT-PCR in additional eight PTC cases. We are now studying a number of genes that we found to be down-regulated in the majority of the PTC tumors, but that have not been previously implicated in PTC. To explore their roles as tumor suppressors, and as candidates in genetic predisposition to this disease, LOH and linkage analysis in PTC kindreds will be done as well as genetic association studies in larger cohorts of PTC patients.

**Gene Expression Profiling of Uterine Leiomyomata.** *M. Mahadevappa<sup>1</sup>, H. Wang<sup>2</sup>, H. Li<sup>2</sup>, Y. Wen<sup>2</sup>, B. Chen<sup>2</sup>, M. Polan<sup>2</sup>, J. Warrington<sup>1</sup>.* 1) Health Management, Applied Research, Affymetrix, 3380 Central Expressway, Santa Clara, CA 95051; 2) Department of Gynecology and Obstetrics, Stanford University School of Medicine, Stanford, CA 94305.

Uterine leiomyomata, fibroid myomas, are common benign smooth muscle tumors occurring in 20-30% of women over 30 years of age. Despite the importance of uterine leiomyomata as a cause of most of the 200,000 hysterectomies performed annually in the United States little is known about its pathogenesis or genetic basis of development. The present study was undertaken to evaluate differences in gene expression in uterine leiomyomata with matched normal myometrium tissue. Labeled target from 8 paired myomas and myometrium was hybridized on GeneChip<sup>®</sup> HuGeneFL arrays containing probes representing approximately 6800 full-length human genes. A list of candidate genes was derived using a combination of analytical tools. Candidate genes identified include genes coding for extracellular proteins, growth factors, hormone/hormone receptors, and mitochondrial transcripts. For a number of genes, results were validated by RT-PCR. These findings will undoubtedly enhance the understanding of the molecular basis of this disease and provide information necessary to design improved diagnostics and therapeutics.



**Immune surveillance genes and breast cancer: does IL6 or TNFa modify BRCA1?** A-M. Martin<sup>1,2</sup>, G.

Athanasiadis<sup>1,2</sup>, P.A. Kanetsky<sup>3</sup>, J.D. Greshock<sup>1,2</sup>, C. Lerman<sup>4</sup>, C. Isaacs<sup>5</sup>, T.R. Rebbeck<sup>3</sup>, B.L. Weber<sup>1,2</sup>. 1) Dept Medicine; 2) Abramson Family Cancer Research Institute; 3) Center for Clinical Epidemiology and Biostatistics; 4) Cancer Center, Univ. of Pennsylvania, Philadelphia, PA; 5) Lombardi Cancer Center, Georgetown University, Washington DC.

Women who carry BRCA1 mutations have up to an 80% risk of developing breast cancer by the time they reach age 70. However, there is considerable variability in the age of diagnosis of breast cancer as well as incomplete penetrance in BRCA1 mutation carriers, suggesting that other factors play a role in BRCA1-associated breast cancer risk. There is evidence that cytokine gene polymorphisms are associated with different diseases, but the exact role of these polymorphisms in cancer is unknown. IL-6 and TNFa play an integral role in the immune response to tumors and are highly polymorphic. Therefore, we sought to determine whether polymorphisms in IL-6 (G-174C) and TNFa (G-238A, G-308A, C-850T, C-856A) were associated with breast cancer occurrence and age of diagnosis among BRCA1 mutation carriers. We studied 222 women with germline BRCA1 mutations, 140 (63%) of whom had breast cancer and 82 (37%) of whom had not been diagnosed with breast cancer. Breast cancer cases were more than twice as likely to carry two G-alleles for the TNF-238A polymorphism (OR=2.5, 95% CI: 0.9, 6.9). Overall, women with breast cancer were only 40% more likely to carry any C-allele for the IL-6-174C polymorphism (OR=1.4, 95% CI: 0.8, 2.4) compared to controls. However, when stratified by age, a significant association was noted in that women diagnosed with breast cancer 50 years of age and older were more than twice as likely to have at least one C-allele (OR=2.3 95% CI: 1.0, 5.1). This association was not seen among younger women (dx<50)(OR=0.72, 95% CI: 0.3, 1.7). We did not find other associations with the remaining 4 loci tested. These preliminary data suggest that IL-6 is associated with an altered risk for breast cancer among older women, and provide the first evidence of a role for immune surveillance genes in breast cancer.

**Stage III gene expression profiles identify a poor prognosis subgroup of stage I lung adenocarcinomas. A.M.**

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The aim of the current study is to determine whether gene expression profiles can be used to identify subgroups of stage I lung adenocarcinomas that more closely resemble stage III tumors, both molecularly and in their overall survival. Gene expression profiles of 87 newly diagnosed lung adenocarcinomas (68 stage I and 19 stage III) were determined using Affymetrix oligonucleotide microarrays, containing 7129 probe sets corresponding to approximately 6800 unique genes. The 7129 probe sets were reduced to the 2469 genes that were consistently expressed at levels high enough for detection by the microarray. To determine which genes were associated with survival in stage III tumors, 2469 univariate Cox proportional hazards models were fit and genes were rank-ordered based on p-values. The top 50 genes significantly associated with the survival of stage III patients (p-value < 0.05) were then used to compute a risk index value for each stage I patient. We define the risk index (RI) as  $RI_i = \sum_j b_j x_{ij}$ , where  $b_j$  corresponds to each of the 50 univariate regression estimates from the stage III patients and  $x_{ij}$  is the gene expression measure for each of these j genes for the ith stage I patient. The median of the distribution of risk indices was used as a cut point to delineate high and low risk stage I patients. Kaplan-Meier analysis showed a statistically significant difference in survival between these high and low risk stage I subgroups (p=0.02). These high and low risk subgroups could not be explained by differences in tumor differentiation, p53, or K-ras mutational status. We also identified statistically significant (p-value < 0.05) mean differences in the expression of 596 genes between high and low risk stage I patients. These results show that gene expression profiles which predict survival in stage III patients may be useful to molecularly discriminate good and poor prognosis stage I patient subgroups. Poor prognosis stage I patients may then benefit from more aggressive therapy beyond surgery.

**Evidence for recurrent deletions spanning the SDHC gene at chromosome 1q21 in families with hereditary paraganglioma type 3 (PGL3).** *J.E. Willett-Brozick<sup>1</sup>, K. Astrom<sup>2</sup>, E.C. Lawrence<sup>2</sup>, R.E. Ferrell<sup>2</sup>, E.N. Myers<sup>3</sup>, B.E. Baysal<sup>1,2,3</sup>.* 1) Dept. Psychiatry, Univ Pittsburgh Med Ctr.; 2) Hum Genet, Univ Pittsburgh; 3) Dept Otolaryngology Univ Pittsburgh Med Ctr, Pittsburgh, PA.

Hereditary paragangliomas (PGL) are benign slow growing tumors of the head and neck. Three loci have been implicated in their pathogenesis: PGL1 at 11q23, PGL2 at 11q13 and PGL3 at 1q21. The genes underlying the PGL1 and PGL3 loci have been identified as SDHD and SDHC, respectively. The SDHD and the SDHC genes encode the small and the large subunits of cytochrome b in mitochondrial complex II. PGL1 is the most common locus and is exclusively transmitted paternally, suggesting genomic imprinting. Many mutations have been described in the SDHD gene which are mainly composed of point mutations, microinsertions and microdeletions, all suggesting loss of function of the protein product. Only a single mutation in SDHC has been uncovered in a PGL3 family. Here, we present mutational analyses of four multiplex PGL families that show no evidence of imprinted transmission. All families excluded linkage to the PGL1 at 11q23, but they showed evidence of linkage to the PGL3 region at 1q21. We performed SSCP and direct sequence analyses of the SDHC gene and failed to identify any mutations. Discovery of several intronic polymorphisms led us to uncover presence of large genomic deletions spanning the SDHC gene in two families. To test whether these two families had the same ancestral mutation we compared their disease chromosome haplotypes. Haplotype analysis did not reveal any similarity between the two families strongly suggesting that the deletions in these families represent independent mutational events. In the other two families large deletions have been excluded by the identification of heterozygosities in the intronic polymorphisms. No intragenic deletions could be identified in any of the four families by RT-PCR analyses. These findings suggest that recurrent large genomic deletions spanning the SDHC gene cause PGL3 and that the pericentromeric region of chromosome 1q21 may contain unstable elements associated with recurrent deletions and other genomic rearrangements.

***CPA3* at 7q32 imprinted domain is a strong candidate gene for prostate cancer aggressiveness.** *T. Kishino*<sup>1,3</sup>, *T. Kayashima*<sup>2,3</sup>, *K. Yamasaki*<sup>2,3</sup>, *S. Hayashida*<sup>2,3</sup>, *N. Miwa*<sup>2</sup>, *N. Niikawa*<sup>2,3</sup>. 1) Nagasaki University Gene Research Center, Nagasaki, Japan; 2) Department of Human Genetics, Nagasaki University School of Medicine, Nagasaki, Japan; 3) CREST, JST, Kawaguchi, Japan.

*MEST/PEG1*, a gene expressed paternally in mesodermal derivatives in early embryonic stages is the first imprinted gene mapped to chromosome 7. Since imprinted genes are clustered in general at a chromosomal region, we speculated that a similar imprinted-gene cluster may exist at 7q32 and that the functions of some such genes may contribute to the phenotype of maternal UPD7. We constructed a complete contig of PAC/BAC clones and transcript map spanning the entire 1-Mb region between *D7S530* and *D7S649* (Hayashida et al., 2000). Based on the Human Transcript Map, we precisely mapped 47 genes/ESTs and analysed the imprinting status of several genes/ESTs adjacent to *MEST* (Yamasaki et al., 2000). Here we report the imprinting status of *CPA3*, carboxypeptidase A3, 200-kb centromeric to *MEST* at 7q32. *CPA3* has been identified as an upregulated gene in PC-3 cells, a prostate cancer cell line, after treatment with sodium butyrate and trichostatin A (TSA), suggesting induction of *CPA3* is mediated by histone hyperacetylation. We analyzed the imprinting status of *CPA3* in fetal tissues and cell lines with and without TSA treatment, which is reported to change expression patterns of some imprinted genes. In the fetal heart, kidney, and lung, *CPA3* revealed a consistent skewed-expression pattern; the maternal derived expression was more abundant than the paternal derived expression. In EB-virus transformed lymphoblastoid cell lines, the expression patterns were variable, i.e., biallelic expression or complete monoallelic maternal expression, and were not changed by TSA treatment. In PC-3 cell line, *CPA3* was biparentally expressed without TSA and upregulated but did not show any skewed expression pattern with TSA. Although this study so far could not demonstrate "loss of imprinting" in prostate cancer, imprinting status of *CPA3* in the prostate may play an important role in prostate cancer aggressiveness, because *CPA3* is just localized in prostate cancer aggressiveness loci reported between markers *D7S3061* and *D7S1804*.

**Association between CYP3A1 gene polymorphisms and the risk for hereditary prostate cancer.** *BL. Chang<sup>1</sup>, SL. Zheng<sup>2</sup>, GA. Hawkins<sup>2</sup>, SD. Isaacs<sup>3</sup>, KE. Wiley<sup>3</sup>, A. Turner<sup>2</sup>, JD. Carpten<sup>4</sup>, ER. Bleecker<sup>2</sup>, PC. Walsh<sup>3</sup>, JM. Trent<sup>4</sup>, DA. Meyers<sup>2</sup>, WB. Isaacs<sup>3</sup>, J. Xu<sup>2</sup>.* 1) University of Maryland School of Medicine, Baltimore, MD; 2) Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC; 3) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD; 4) National Human Genome Research Institute, National Institute of Health, Bethesda, MD.

The cytochrome P450 3A (CYP3A) genes play an essential role in steroid and drug metabolism, and are candidate genes for prostate cancer. Five processed CYP3A genes: 3A3, 3A4, 3A5, 3A7, 3A43, and two pseudogenes: 3AP1 and 3AP2, are all mapped to 7q21-q22. An allelic variant, CYP3A4V has been shown to be associated with advanced prostate cancer. The 3AP1-t369g polymorphism was found to be associated with increased CYP3A5 activity. To evaluate the respective role of CYP3A genes in hereditary and sporadic prostate cancer, we performed linkage and association studies in 159 hereditary prostate cancer (HPC) families, 249 sporadic prostate cancer cases, and 211 unaffected controls. No significant evidence for linkage was observed in the region (peak LOD assuming heterogeneity (HLOD)=0.10 at D17S1522), although the HLODs were higher in the subset of HPC families with 4 affected relatives (peak HLOD=1.03 at D17S1522). The frequencies of six SNPs in CYP3A4, 3A5, and 3AP1 were compared between the probands, sporadic cases, and unaffected controls. All the SNPs were in Hardy-Weinberg Equilibrium and all the pair-wise linkage disequilibrium tests were highly significant ( $p < 0.00001$ ). No association between CYP3A4V and the risk of prostate cancer was observed. However, increased frequencies of rare alleles were observed in HPC probands and sporadic cases, compared with unaffected controls for all the SNPs in the 3A5 and 3AP1 genes. The frequency of heterozygotes of 3AP1-t369g in HPC probands (24%) was significantly higher compared with controls (15%) (OR=1.98, 95% CI= 1.11-3.55,  $p=0.02$ ). This difference was larger in the older age of onset group. Our results suggest that sequence variants in the CYP3A5 may increase prostate cancer risk, especially in HPC.

**Mutation screen and association test of 1-a-hydroxylase gene in prostate cancer.** GA. Hawkins<sup>1</sup>, SL. Zheng<sup>1</sup>, S. Cramer<sup>1</sup>, SD. Isaacs<sup>2</sup>, KE. Wiley<sup>2</sup>, BL. Chang<sup>3</sup>, J. Mychaleckyj<sup>1</sup>, JD. Carpten<sup>4</sup>, ER. Bleecker<sup>1</sup>, PC. Walsh<sup>2</sup>, JM. Trent<sup>4</sup>, DA. Meyers<sup>1</sup>, WB. Isaacs<sup>2</sup>, J. Xu<sup>1</sup>. 1) Center for Human Genomics, Wake Forest Univ School of Medicine, Winston-Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD; 3) University of Maryland School of Medicine, Baltimore, MD; 4) National Human Genome Research Institute, National Institute of Health, Bethesda, MD.

1-a, 25 dihydroxyvitamin D3 (1,25(OH)2D3) is the active hormonal form of vitamin D3. Because 1,25(OH)2D3 exhibits potent antiproliferative properties on normal and neoplastic prostatic cells, it has been proposed that 1,25(OH)2D3 may have a role in regulating the growth of prostatic cancer cells. Thus any defects in the metabolic pathway of 1,25(OH)2D3 production could be implicated in increased risk for prostate cancer. The enzyme 1-a-hydroxylase catalyzes the final step of the vitamin D anabolic cascade, to convert the vitamin D prohormone 25-hydroxyvitamin D3 (25 OH)D3) to the active hormonal metabolite 1,25(OH)2D3. To test the hypothesis that sequence variants in the 1-a-hydroxylase gene may be associated with prostate cancer risk, we sequenced the entire 1-a-hydroxylase gene (exons and introns) in 96 individuals, including 72 Caucasians and 24 African Americans. Each racial group consisted of equal numbers of hereditary prostate cancer cases, sporadic cases, and unaffected controls. One SNP in the coding region was identified, and did not change an amino acid. Five other SNPs were also identified in the non-coding region. Interestingly, the four coding SNPs that had previously been identified and associated with pseudovitamin D deficiency (rickets) were not observed in the 96 samples. We then genotyped four frequent SNPs in a larger set of cases and control samples (total 249 cases and 222 controls). All genotyped SNPs were found to be in Hardy-Weinberg equilibrium and in strong linkage disequilibrium ( $P < 0.00001$ ). No significant difference was found in the allele and genotype frequencies between the sporadic cases and unaffected controls. Our results suggest that the 1-a-hydroxylase gene does not play a major role as a prostate cancer susceptibility gene.

**Mutation screen and association test of DLC1 gene in hereditary and sporadic prostate cancer patients. S.**

Zheng<sup>1</sup>, GA. Hawkins<sup>1</sup>, J. Mychaleckyj<sup>1</sup>, SD. Isaacs<sup>2</sup>, KE. Wiley<sup>2</sup>, AR. Turner<sup>1</sup>, BL. Chang<sup>3</sup>, JD. Carpten<sup>4</sup>, ER. Bleecker<sup>1</sup>, PC. Walsh<sup>2</sup>, JM. Trent<sup>4</sup>, DA. Meyers<sup>1</sup>, WB. Isaacs<sup>2</sup>, J. Xu<sup>1</sup>. 1) Center for Human Genomics, Wake Forest Univ. Sch. of Med., Winston-Salem, NC; 2) Department of Urology, Johns Hopkins Medical Institutions, Baltimore, MD; 3) University of Maryland School of Medicine, Baltimore, MD; 4) National Human Genome Research Institute, National Institute of Health, Bethesda, MD.

A gene or genes on chromosome 8p22-23 have been implicated in prostate carcinogenesis by the observation of frequent deletions of this region in prostate cancer cells, and by two recent linkage studies in hereditary prostate cancer (HPC) families. To identify prostate cancer susceptibility genes, we are currently screening candidate genes in the region, including DLC1 (deleted in liver cancer). DLC1 is a candidate tumor suppressor gene because of its 86% homology with rat p122 RhoGAP, which catalyzes the conversion of the active GTP-bound rho complex to the inactive GDP-bound form, suppressing Ras-mediated oncogenic transformation. A previous study identified a missense mutation and three intronic insertions/deletions in 126 primary human colorectal tumors. However, no mutation screening for DLC1 in prostate tumor or germ line DNA of prostate cancer patients has been previously reported. In this study, we report the results of a mutation screen and association test of DLC1 in hereditary and sporadic prostate cancer genomic DNA samples. The PCR products of all 14 exons, exon-intron junctions, and 3' UTR were directly sequenced in 159 HPC probands. Six SNPs were identified in the coding regions; all are conservative changes. In addition, 25 other SNPs were identified in the non-coding regions. Nine of the 31 SNPs were frequent (>10%) and thus were genotyped in 249 sporadic cases and 222 unaffected controls. All the genotyped SNPs were in Hardy-Weinberg Equilibrium, and all the pair-wise SNPs were in strong LD ( $P < .00001$ ). No significant difference in the allele and genotype frequencies were observed among HPC probands, sporadic cases, and unaffected controls. These results suggest that DLC1 is unlikely to be an important prostate cancer susceptibility gene.

**Analysis of linkage disequilibrium in Caucasian and African-American breast cancer cases and controls reveals different evolutionary histories of BRCA1 vs BRCA2.** *T. Walsh<sup>1</sup>, R.K. Kim<sup>1</sup>, B. Newman<sup>2</sup>, M-C. King<sup>1</sup>.* 1) Depts of Medicine and Genomic Sciences, University of Washington, Seattle, WA; 2) School of Public Health, Queensland University of Technology, Australia.

In order to understand the genomic structure and evolution during human history of BRCA1 and BRCA2, we identified multiple SNPs (with rarer allele frequency 0.2 or higher) to span 80 kb of the coding, intronic, and regulatory sequences of each gene. All SNPs were genotyped for a series of 800 breast cancer cases and matched controls, of European and African-American ancestry, from the population-based Carolina Breast Cancer Study. Frequencies of individual SNPs were the same in cases vs. controls, and allele frequencies of all SNPs were in Hardy-Weinberg equilibrium. However, linkage disequilibrium (LD) relationships differed between BRCA1 and BRCA2 and between haplotypes by continent of origin (African-Americans were assumed to carry haplotypes of both African and European origins). Results suggest very different histories of the two genes. At BRCA1, LD across the locus is 1.0 in each population. However, at BRCA1 differences in haplotype frequencies between populations suggest a founder effect and/or selection on BRCA1 after migrations to Europe. In contrast, at BRCA2, no dramatic European-specific selection is suggested. Rather, at BRCA2, LD varies across the locus, with patches of low LD embedded in regions of overall high LD. This pattern could reflect the existence of highly mutable sites. How these different histories may be associated with persistence or dispersion of cancer-predisposing alleles is still not clear to us.



**Linkage analysis in a collection of German PCA families reveals a high contribution of HPCX.** *W. Vogel<sup>1</sup>, T. Paiss<sup>2</sup>, K. Herkommer<sup>2</sup>, J. Haeussler<sup>1</sup>, S. Bochum<sup>1</sup>.* 1) Dept Human Genetics, Univ Ulm, Ulm, Germany; 2) Dept Urology, Univ Ulm, Ulm, Germany.

Obviously there are considerable differences in allele frequencies of different prostate cancer susceptibility genes between different populations yielding variable results in linkage analyses. Therefore it seems worthwhile to establish independent family collections in different countries and to assess the contribution of the different loci in these populations. We have now a collection of 222 German families (3.2 affecteds per family, and 64.9 years mean age at onset) with 104 families used here, which is a sufficiently large sample to allow for linkage studies. HPCX is one of the 5 first PCa susceptibility loci and its contribution to familial PCa had been found highly different in US populations compared to Finland (14% versus 33%). In the 104 German families analysed, we found a maximum HLOD of 1.65 and a 0.34 in the subset of 63 families that had a mean age at onset < 65 years. The corresponding NPL Z score was 2.32 ( $P = 0.009$ ) at DXS984, formally confirming the original linkage. The position of the highest LOD score is close to that of earlier studies (7cM from DXS1113). Preliminary results on the 3 loci on chromosome #1 indicate that these play only a minor role in familial PCa in Germany (highest NPL Z score 1.18 at D1S2785 in the PCaP locus, HLOD < 1 and a < 0.2 throughout). Our results confirm linkage to HPCX and are very similar to those in the Finnish families. This similarity may indicate a rather homogeneous situation in Europe. The difference to the caucasian US population remains unexplained and is difficult to understand, given the very recent European origin of this population.

**Tumour specific promoter methylation of the human homologue of the drosophila Roundabout gene DUTT1**

**(ROBO1) in human cancers** . A. Dallot<sup>1</sup>, E. Forgacs<sup>2</sup>, A. Martinez<sup>1</sup>, Y. Sekido<sup>2</sup>, P. Rabbitts<sup>3</sup>, E.R. Maher<sup>1,4</sup>, J.D. Minna<sup>2</sup>, F. Latif<sup>1,4</sup>. 1) Department of Paediatrics and Child Health, University of Birmingham, UK; 2) Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Texas, USA; 3) Medical Research Council Centre, Hills Road, Cambridge, UK; 4) CRC Renal Molecular Oncology Research Group, University of Birmingham, UK.

The human homologue of the drosophila Roundabout gene DUTT1 (Deleted in U Twenty Twenty) or ROBO1, a member of the NCAM family of receptors, was recently cloned from the lung cancer tumour suppressor region 2 (LCTSGR2 or U2020 region) at 3p12. DUTT1 overlaps homozygous deletions characterised in both small cell lung cancer lines (SCLC) and in a breast cancer line. We(a) defined the genomic organisation of DUTT1 gene, (b) performed mutation and expression analysis of DUTT1 in lung, breast and kidney cancers, (c) identified tumour specific promoter region methylation of DUTT1 in human cancers. The gene was found to contain 29 exons and spans at least 240 kb of genomic sequence. The 5' region contains a CpG island, and the poly A tail has an atypical GATAAA signal. We analysed DUTT1 for mutations in lung, breast and kidney cancers, however, no inactivating mutations were detected by PCR-SSCP. Seven germline missense changes were found and characterised. DUTT1 expression was not detectable in 1/14 breast tumour lines analysed by RT-PCR. Bisulphite sequencing of the promoter region of DUTT1 gene in the HTB-19 breast tumour cell line showed partial hypermethylation of CpG dinucleotides. The expression of DUTT1 was restored after demethylating with 5-aza-2-deoxycytidine. The same region was also found to be hypermethylated in 6/32 (19%) primary breast tumours and 8/44 (18%) primary clear cell renal cell carcinomas (CC-RCC) and in 1/26 (4%) primary NSCLC tumours. Furthermore 80% of breast and 75% of CC-RCC tumours showing DUTT1 methylation had allelic losses for 3p12 markers hence obeying Knudsons two hit theory. These findings suggest that DUTT1 should be considered as a candidate for the TSG at 3p12.

**RASSF1A promoter region CpG island hypermethylation in pheochromocytoma and neuroblastoma tumors. A.**

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Deletions of chromosome 3p are frequent in many types of neoplasia including neuroblastoma (NB) and pheochromocytoma. Recently we isolated several candidate tumour suppressor genes (TSGs) from a 120kb critical interval at 3p21.3, defined by overlapping homozygous deletions in lung and breast tumor lines. A CpG island in the promoter region of one of the genes (RASSF1A) was found to be methylated in a majority of lung tumors. To investigate the role of 3p TSGs in neural crest tumors, we (a) analyzed 41 pheochromocytomas for allele loss in regions proposed to contain 3p TSGs, (b) examined 23 sporadic pheochromocytomas for evidence of RASSF1A methylation and (c) investigated 67 neuroblastoma tumors for RASSF1A inactivation. We found that 46% of pheochromocytomas showed allele loss at one or more 3p marker(s), and 38.5% demonstrated loss for 3p21.3 marker(s). RASSF1A promoter region hypermethylation was found in 22% (5/23) of sporadic pheochromocytomas and in 55% (37/67) of neuroblastomas analyzed but RASSF1A mutations were not identified. In a neuroblastoma cell line, methylation of RASSF1A correlated with loss of RASSF1A expression and RASSF1A expression was restored after treatment with the demethylating agent 5-azacytidine. These results indicate that RASSF1A inactivation by hypermethylation is important in neural crest tumorigenesis, particularly neuroblastoma.

**Epigenetic inactivation of the RASSF1A tumour suppressor gene (TSG) in renal cell carcinoma.** *C. Morrissey<sup>1</sup>, A. Martinez<sup>1</sup>, M. Zatyka<sup>1</sup>, A. Agathangelou<sup>1</sup>, P. Schraml<sup>2</sup>, T. Kishida<sup>3</sup>, F. Richards<sup>1</sup>, F. Latif<sup>1</sup>, E. Maher<sup>1</sup>.* 1) Medical and Molecular Genetics, University of Birmingham, Birmingham, West Midlands, UK; 2) University of Basel, Switzerland; 3) Yokohama City University, Japan.

Renal cell carcinoma (RCC), the most common adult kidney neoplasm, is histopathologically heterogeneous, with most sporadic RCC (~80%) classified as clear cell tumours (cc-RCC). Chromosome 3p allele loss is the most frequent genetic alteration in RCC, but is associated specifically with CC-RCC and is not a feature of non-clear cell RCC such as papillary. Somatic inactivation of the VHL TSG located at chromosome 3p25 occurs in up to 70% of CC-RCC tumours and cell lines. However VHL inactivation is not sufficient for CC-RCC tumourigenesis and inactivation of 3p12-p21 TSG(s) appears necessary irrespective of VHL gene status. Recently we demonstrated that the candidate 3p21 TSG, RASSF1A, is hypermethylated in most small cell lung cancers. We have now investigated the role of RASSF1A inactivation in primary RCC tumours. RASSF1A promoter methylation was detected in 23% of primary CC-RCC tumours. In CC-RCC cell lines RASSF1A methylation was associated with silencing of RASSF1A expression which was restored after treatment with 5azacytidine. The frequency of RASSF1A methylation was similar in CC-RCC with and without VHL gene inactivation (24%/29%), with no association between epigenetic silencing of the RASSF1A and VHL TSGs. Although 3p allele loss is reported rarely in papillary RCC, we identified RASSF1A methylation in 44% of papillary RCC analysed. Thus (a) inactivation of RASSF1A is frequent in both CC-RCC and papillary RCC tumours and (b) there is no relationship between epigenetic silencing of RASSF1A and VHL in CC-RCC. 54 CC-RCC analysed for RASSF1A methylation were informative for 3p21 allele loss, of which 20% demonstrated RASSF1A methylation. All informative CC-RCC with 3p21 loss and no RASSF1A methylation demonstrated allele losses elsewhere in 3p so in these cases tumourigenesis may result from (a) haploinsufficiency of RASSF1A, (b) inactivation of other 3p21 TSGs or (c) inactivation of TSGs outside of 3p21. RASSF1A is the first TSG to be inactivated frequently in both papillary and CC-RCC.

**Allele-specific methylation of the NF1 gene during development.** *T.R. Haines<sup>1</sup>, D.R. Rodenhiser<sup>1</sup>, P.J. Ainsworth<sup>1,2</sup>.*  
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Cytosine methylation is the principal base modification of vertebrate DNA. In adult tissues 5-methylcytosines are found predominantly in CpG dinucleotides, although non-CpG methylation exists in cultured murine embryonic stem cells, mainly at CpA dinucleotides. Parent-of-origin effects observed in de novo mutagenesis at the human neurofibromatosis (NF1) gene locus have led us to examine methylation patterns of the highly homologous murine Nf1 gene, both in gametes and at the earliest stages of preimplantation development. Point mutations, which form the majority of de novo NF1 mutations, have been shown to be primarily of paternal origin, while the less common larger deletion events are frequently maternally derived. We have evaluated the methylation status of a CpG dinucleotide within the murine Nf1 gene that correlates with a site of recurrent mutation (C5839T) in the human NF1 gene. Using bisulfite genomic sequencing we have shown that this CpG site was fully methylated in sperm, oocyte and in the early preimplantation embryo, with no allele-specific bias being seen. Unexpectedly, we have discovered allele-specific non-CpG methylation at the Nf1 locus, found primarily at CpA dinucleotides and to a lesser extent at CpTs. Non-CpG methylation at the Nf1 locus was specific to oocyte DNA and to the maternal allele of the 2-cell embryo. This pattern of non-CpG methylation was not found in DNA from sperm or in the paternally-derived allele of the 2-cell embryo. Non-CpG methylation was temporally restricted to early embryogenesis, and did not persist beyond the 8-cell stage of development. This non-CpG methylation may be a more widespread event in the early embryo since we have also observed the presence of similar non-CpG methylation at the murine adenosine deaminase gene in 2-cell embryos. We speculate that these transient patterns of non-CpG methylation may be related to the parent-of-origin effects seen in de novo mutation events in the NF1 (and possibly other) gene loci. As well, this type of allele-specific methylation may play a part in the control of wider patterns of gene expression in the early stages of preimplantation development.*de novo.*

**Comprehensive DNA methylation study in 40 uveal melanomas.** *M. Zeschnigk, B. Horsthemke, D.R. Lohmann.*  
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Uveal melanoma is the most common form of primary eye cancer. Monosomy 3, which is an unusual finding in tumors, but present in about 50% of uveal melanomas, is significantly correlated with metastatic disease. Long-term studies have shown that 4 years after diagnosis approximately 70% of patients showing monosomy 3 in the primary tumor have died of metastases, whereas tumors with disomy 3 rarely give rise to metastatic disease. In many tumors, recurrent loss of genetic material is part of a two step inactivation of tumor suppressor genes. Inactivation of second alleles might be caused by local mutations such as point mutations, gene deletions or epigenetic silencing. The observation of isodisomy of chromosome 3 in some uveal melanomas has led to the hypothesis that epigenetic factors might play a role in tumor development and metastatic disease. We have performed a comprehensive methylation study of the promoter regions of 8 genes reported to be involved in tumorigenesis. Five of these genes are located on chromosome 3. Additionally, the SNRPN gene located in the the imprinted region on chromosome 15 was included in the study. Using the bisulfite genomic sequencing protocol or the methylation specific PCR (MSP) the methylation patterns of these genes were analyzed in 20 uveal melanomas with monosomy 3 and in 20 uveal melanomas with disomy 3. None of the tumors showed hypermethylation in the promoter regions of the fragile histidine triad (FHIT), beta-catenin, von Hippel-Lindau (VHL), E-cadherin, activated leukocyte cell adhesion molecule (ALCAM), and retinoblastoma (RB1) genes. Three tumors showed some hypermethylation in the retinoic acid receptor (RAR) promoter on chromosome 3, and in 3 other tumors the p16 gene promoter region was hypermethylated. The imprinted SNRPN and PW71 loci were hypermethylated in 3 out of 40 tumors. We conclude that methylation in the analyzed genes occurs infrequently in uveal melanoma and appears not to be associated with the chromosome 3 status.

**Epigenetic inactivation of the *RASSF1A* tumour suppressor gene in Wilms' tumour.** *K.J. Wagner<sup>1</sup>, W.N. Cooper<sup>1</sup>, R.G. Grundy<sup>1,2</sup>, R. Wadey<sup>1,2</sup>, F. Latif<sup>1</sup>, E.R. Maher<sup>1</sup>.* 1) Medical and Molecular Genetics, University of Birmingham, Birmingham, West Midlands, United Kingdom; 2) Department of Paediatric Oncology, Birmingham Childrens Hospital, Birmingham, West Midlands, United Kingdom.

Promoter methylation is an important mechanism of tumour suppressor gene (TSG) inactivation in human cancer. Although studies of classical TSGs such as *VHL* and *RBI* suggested that TSG inactivation by promoter methylation is less frequent than somatic mutations, recently epigenetic silencing has been established as the major mechanism of inactivation of the 3p21.3 TSG, *RASSF1A*. We have established that *RASSF1A* methylation is frequent in both clear cell and papillary adult renal cell carcinomas, even though 3p21.3 allele loss is rare in papillary tumours. Wilms tumour is the most common childhood kidney tumour and is thought to be derived from primitive nephroblasts. Somatic mutations in the *WT1* TSG can be detected in ~10% of Wilms tumours and although epigenetic alterations at *IGF2* and *H19* are common, inactivation of TSGs such as *p16* and *p53* occur in only a minority of Wilms tumours so the identification of major Wilms TSGs is a priority. Although 3p allele loss is infrequent in Wilms tumour, in view of its involvement in adult renal cancers we investigated *RASSF1A* as a candidate Wilms TSG. We detected *RASSF1A* hypermethylation by methylation-specific PCR (MSP) in 20 of 40 (50%) primary Wilms tumours. *RASSF1A* methylation was detected in normal tissue DNA for only 2 of 14 samples analysed (in both of these cases the corresponding tumour demonstrated *RASSF1A* methylation). We investigated the relationship between *RASSF1A*, *p16* and *H19* methylation in Wilms tumour but did not find a statistically significant association. We have demonstrated that epigenetic inactivation of *RASSF1A* is the most frequent event in Wilms tumourigenesis and that in specific tumours types a major TSG may exist in regions with a low frequency of allele loss.

**Polyomavirus Large T Antigen / MMTV Transgenic Mice as a Model of Uterine Leiomyomata.** *D.M. Neskey*<sup>1</sup>, *A.H. Ligon*<sup>2,3</sup>, *B.J. Quade*<sup>2,3</sup>, *C.C. Morton*<sup>1,2,3</sup>. 1) Dept Obstetrics, Gynecology, and Reproduction Biology, Brigham & Women's Hospital, Boston, MA; 2) Dept Pathology, Brigham & Women's Hospital, Boston, MA; 3) Harvard Medical School, Boston, MA.

Uterine leiomyomata, commonly known as fibroids, are benign smooth muscle tumors found in up to 70% of reproductive-aged women and are the cause of more than 200,000 hysterectomies performed annually in the United States. To study further the biology of these tumors and to aid in the design of nonsurgical therapies, we have characterized a transgenic mouse model (MMTV/PyVLT) expressing the polyomavirus (PyV) large T (LT) antigen driven by the mouse mammary tumor virus enhancer / promoter (Oncogene 16:1963-1972, 1998). Although a number of animal models are currently being studied in which uterine tumors are present no single model fully represents the human condition and many biological questions await evaluation. Uterine tumors were observed in 22/46 MMTV/PyVLT transgenic females and were negatively correlated with the parity of the animal. Tumors were observed as early as 6 months of age in 1/3 nulliparous mice. Histological study of these neoplasias suggested similarities with their human counterpart, including a spindle-cell appearance in culture and a generally cytologically normal appearance. Interestingly, mitotic activity varied from <1 to >10 mitotic figures per 10 HPF. Necrosis was limited to the core in most tumors, but occasionally was more extensive and geographic. Cellularity also varied from low (with increased amount of intracellular matrix) to high (with virtually no intracellular matrix), and generally correlated with mitotic activity. Cytogenetically, 40% of human leiomyomata show karyotypic abnormalities, but initial analyses of four murine tumors to date have not revealed any clonal chromosomal changes. Future studies will involve identification and characterization of orthologous genes and proteins involved in the pathogenesis of human uterine leiomyomata (i.e., HMGA1 and HMGA2) using microarray and tissue array technologies.



**Phenotypic characteristics of colon cancer in I1307K APC germline mutation carriers compared with sporadic cases.** *E. Friedman*<sup>1</sup>, *A. Figer*<sup>2</sup>, *L. Irmin*<sup>1</sup>, *R. Geva*<sup>2</sup>, *A. Tamir*<sup>3</sup>. 1) Oncogenetics Unit, Inst Gen, Chaim Sheba Medical Ctr, Tel Hashomer, Israel; 2) Institute of Oncology, Sourasky Medical Center, Tel-Aviv, Israel; 3) epidemiology Unit, Hacarmel Medical Center, Haifa, Israel.

The I1307K APC germline mutation is associated with an increased risk to colorectal cancer (CRC). Whether and to what extent the phenotype of CRC in mutation carriers differs from sporadic cases, remains unknown. To gain insight into this issue, we analyzed 307 unselected Israeli patients with CRC, who were treated in a single medical center, for harboring the I1307K mutation. Twenty-eight mutation carriers (9.1%) were detected. Two of 28 mutation carriers (7.1%) and 93/277 (33.6%) of non-carriers, were of non-Ashkenazi origin ( $p < 0.01$ ). In 74/278 (26.6%) of the sporadic cases, and only 1/28 (3.6%) of mutation carriers (3.6%) the tumor was located in the right colon ( $p < 0.01$ ). Mutation carriers had a more advanced disease stage (14/28 50% Dukes C), as compared with 60 (19.5%) of non-carriers ( $p = 0.02$ ). The mean age at diagnosis was similar: 65 (+/- 9.7) years and 66.3 (+/- 11.6) years, for mutation carriers and non-carriers, respectively. No statistical differences were noted between the two groups in sex distribution, tumor grade, and family history of cancer. We conclude that early age at diagnosis and family history of cancer are not consistent features in I1307K APC germline mutation carriers, and with the possible exception of a more advanced disease stage, this mutation probably has little effect on the phenotypic features of CRC.

**Comprehensive mutation scanning of ATM gene in patients with breast cancer reveals a higher frequency of missense mutations in breast cancer relative to ethnically matched controls.** *J. Feng<sup>1</sup>, C.H. Buzin\*<sup>1</sup>, M. Jung\*<sup>2</sup>, J. Zheng<sup>1</sup>, Q. Liu<sup>1</sup>, S.J. Jeong<sup>2</sup>, J. Moulds<sup>2</sup>, V.Q. Nguyen<sup>1</sup>, W.P. Bennett<sup>1</sup>, A. Dritschilo<sup>2</sup>, S.S. Sommer<sup>1</sup>.* 1) Dept. of Molecular Genetics, City of Hope Medical Center, Duarte, CA; 2) Dept. of Radiation Medicine, Georgetown University Medical Center, Washington, D.C. \*These authors contributed equally to this work.

Epidemiological studies have shown an increased risk of breast cancer in obligate heterozygote carriers among family members of ataxia telangiectasia (A-T) patient. However, the expected increase in levels of ATM gene mutations among unselected breast cancer patients has not been found to date. Previous methods of mutation detection were biased toward the detection of truncating mutations, and single nucleotide substitutions were likely to have been under-reported. In this study, genomic DNAs from 43 breast cancer patients and 43 controls were scanned in the entire ATM coding region (exons 4-65) and adjacent intronic splice regions using DOVAM-S (Detection Of Virtually All Mutations-SSCP), a modification of SSCP in which there is sufficient redundancy to detect virtually all mutations. Excluding D1853N found commonly in cases and controls, there were one protein truncating mutation and 14 missense changes in the cases vs. 8 missense changes in controls ( $p=0.06$ ). When all structural changes common to cases and controls were excluded, ten missense changes and one splice mutation were found in cases and two missense changes in controls ( $p=0.013$ ). This is, to our knowledge, the first study to comprehensively scan the ATM gene in control samples of known ethnicity. The background of missense changes in controls is high. There was a trend towards elevation of all structural changes in cases, but the results are not statistically significant. Cohort-specific structural changes were significantly more prevalent in the breast cancer patients. The data are compatible with certain missense mutations in ATM predisposing to breast cancer. If the results can be confirmed in additional studies, it may be of interest to determine whether women with certain missense mutations in the ATM gene respond better to radiation therapy or radiomimetic chemotherapy.

**P53 mutation analysis of breast tumors from a large unselected cohort of BRCA2 mutation carriers and non carriers.** *S. Gudlaugsdottir<sup>1</sup>, H. Hilmarsdottir<sup>1</sup>, J.G. Jonasson<sup>2</sup>, S. Thorlacius<sup>1</sup>, G. Olafsdottir<sup>1</sup>, L. Tryggvadottir<sup>1</sup>, H.M. Ogmundsdottir<sup>1</sup>, J.E. Eyfjord<sup>1</sup>.* 1) Icelandic Cancer Society; 2) University Hospital of Iceland.

It has been postulated that somatic mutations in the p53 gene or other DNA-damage signaling components, are necessary to give a growth advantage to cells with BRCA1 and BRCA2 mutations allowing the transformation of cells or the development of tumors. Consistent with this idea, our previous results and those of others have suggested that there is a higher frequency of p53 mutation in carriers of BRCA germline mutations. In the current study we have enlarged a study group from 402 unselected breast cancer cases to 1301. In our previous study, the p53 mutation frequency, testing exons 5-8, was 29% in BRCA2 carriers and 17% in non-carriers but in the current study the mutation rates are 23% in carriers and 18% in non-carriers. This indicates that the difference between the two groups is less than previously reported. The p53 mutation frequency in the group as a whole is the same, or slightly higher (18.4% as compared to 18.0%), than in the earlier study and the exon distribution is similar in both groups. According to this study there is a slightly increased frequency of p53 mutations in breast tumors from carriers of a BRCA2 999del5 germ line mutation as compared to non-carriers. The difference, however, is not statistically significant. These results differ from published data which are largely based on tumors from BRCA1 mutation carriers. As there is evidence indicating that in tumors with BRCA germline mutation, changes are common at p53 codons that are not mutation hotspots, sequencing of the entire p53 gene is now in progress. The results are also being analysed with respect to clinico-pathologic parameters.

**BRCA1 and BRCA2 founder mutations in Spanish and Mexican populations.** *M. Alvarez-Franco<sup>1</sup>, G. Llorc<sup>2</sup>, R. Gerson<sup>3</sup>, A.E. Bale<sup>1</sup>*. 1) Yale University School of Medicine, New Haven, CT; 2) Instituto Catal(á)n de Oncologia, Barcelona, Spain; 3) Hospital General de M(é)xico, DF. M(é)xico.

BRCA1 and BRCA2 account for a substantial proportion of all hereditary breast cancer. Hundreds of different BRCA mutations have been reported worldwide, but the number of different mutations present in any specific ethnic group may be limited. The Mexican population is represented mainly by Mestizos, a mixture of Spanish and Native American. The Spanish gene pool is derived from a relatively small group of Conquistadores and early colonists who arrived in the region that is now Mexico in 16th century. Based on this history, a subset of the BRCA mutations found in Spain could be present as high frequency founder mutations in Mestizos. We analyzed 77 Spanish breast/ovarian cancer families for mutations in BRCA1 and BRCA2 and identified novel mutations in 14 families and recurrent mutations in 5 families- 185delAG (family not known to be of Jewish ancestry), 189insTGTC, 5537delA and two families with 9254delATCAT. Combining our results with other published data, a total of 43 unique and 5 recurrent mutations have been reported in Spanish families. All of these mutations can be detected by sequencing 21 exons of BRCA1 and 2. We obtained DNA samples from 73 Mexican women with familial breast/ovarian cancer and have so far tested 5 families for the mutations found in Spain. A Spanish mutation in BRCA1 (Arg71Gly) predicted to alter splicing, was identified in one family. This mutation was reported before in an Hispanic family from the Caribbean region and may prove to be a common founder mutation among Hispanics. Additional sequencing of the Mexican population is in progress.

**A role of MLH3 in hereditary nonpolyposis colorectal cancer.** *R.M.W. Hofstra<sup>1</sup>, Y. Wu<sup>1</sup>, M.J.W. Berends<sup>2</sup>, R.H. Sijmons<sup>1</sup>, R.G.J. Mensink<sup>1</sup>, E. Verlind<sup>1</sup>, K.A. Kooi<sup>1</sup>, T. van der Sluis<sup>3</sup>, C. Kempinga<sup>3</sup>, A.G.J. van der Zee<sup>4</sup>, H. Hollema<sup>3</sup>, J.H. Kleibeuker<sup>2</sup>, C.H.C.M. Buys<sup>1</sup>.* 1) Dept. Medical Genetics, University of Groningen, Groningen, The Netherlands; 2) Dept. Gastroenterology, University of Groningen, Groningen, The Netherlands; 3) Dept. Pathology, University of Groningen, Groningen, The Netherlands; 4) Dept. Gynecology, University of Groningen, Groningen, The Netherlands.

The recently cloned mismatch repair (MMR) gene MLH3 might well be involved in the pathogenesis of hereditary nonpolyposis colorectal cancer (HNPCC) because it is associated with mammalian microsatellite instability (MSI) and because its product interacts with MLH1, another MMR protein involved in HNPCC. To investigate a possible involvement of MLH3 in HNPCC we scanned the 12 exons of the gene for mutations in index patients of 39 HNPCC families and in 288 patients suspected for HNPCC. Eleven different germline MLH3 variants, one frameshift and ten missense mutations, were identified in 2 members of genuine HNPCC families and 17 patients with HNPCC suspicion. From 16 of these 19 patients a total of 18 tumours were available for microsatellite analysis. Only five of the 18 tumours displayed MSI-H phenotype at the five consensus markers. Using a different set of five markers not including mono but di and tetranucleotide repeats, 16 of the 18 tumours exhibited an MSI-H phenotype, suggesting an MLH3-specific MSI profile. Five of the 19 patients with an MLH3 variation also carried a mutation in another MMR gene, four in MSH6 and one in MLH1. In some cases, it seems, therefore, that different mutant genes need to occur in combination in order to predispose to tumour development.

**Is homozygosis for the C677T mutation of the MTHFR gene a protective factor for sporadic breast cancer?** *O.M. Mutchinick, L. García, M.A. López, J. Arteaga.* Dept de Genética, Inst Nac de Ciencias Médicas y Nutrición Salvador Zubirán, D.F., México.

Breast cancer (BC) is the first cause of women cancer in the USA and the second in México. Genetic, environmental and life style determinants have been frequently associated to BC. It appears that the MTHFR C@T mutation modulates carcinogenesis by decreasing the risk for CRC and ALL and increasing the risk for endometrial and BC. Apparently, the protective effect is folate dependent. Studies from our group demonstrated a very high prevalence of the T allele (56-81%) and TT genotype (35-68%) and a low folic acid (FA) intake in diverse Mexican *mestizo* and Indian populations.

**Objevtive:** to evaluate the genetic-nutrition risk interaction for BC of the MTHFR C677T mutation and the daily intake of FA, B2, B6 and B12 vitamins. **Design:** prolective case-control study. **Participants:** 78 women with sporadic BC, 71 patients with sporadic CRC and 250 healthy females (HF) from different parts of the country. **Methods:** identification of the C@T mutation by PCR and restriction enzyme analysis and FA, B2, B6 and B12 ingestion by a food frequency intake questionnaire.

**Results:** BC patients showed a significantly lower prevalence of the TT genotype (20.5%) than CRC (38.0%) and HF (34.8%) with gene frequencies for the mutant allele of 44.8, 59.2 and 58.6% respectively. All groups were in H-W equilibrium. Comparison among groups only showed statistical differences (SD) between BC and CRC, OR:0.35 (0.13-0.93),  $p=0.03$ , and between BC and HF, OR:0.34 (0.15-0.74),  $p=0.005$ . SD were also observed for FA ( $p=0.016$ ) and B2 ( $p=0.03$ ) in BC, with a higher intake of both vitamins in TT than CC patients. Similar SD was also found for comparisons between BC-CRC and BC-HF subjects with a TT genotype.

**Conclusions:** Against others finding, our results strongly suggest that TT women have an important lower risk for sporadic BC. That this protective effect of the TT genotype appears to be folate and B2 dependent. Our data also confirm the important role recently attributed to B2 in folate metabolism and MTHFR thermolabile variant activity.

**Germline mutations in CDH1/E-Cadherin in families with hereditary prostate-gastric cancer.** *B.-A. Jonsson<sup>1</sup>, A. Bergh<sup>1</sup>, M. Emanuelsson<sup>2</sup>, H. Gronberg<sup>2</sup>.* 1) Medical Biosciences, Pathology, Umeå University, Sweden; 2) Department of Radiation Sciences, Oncology, Umeå University, Sweden.

Germline mutations of the E-cadherin gene (CDH1) have been found in families with hereditary diffuse gastric cancer. In total, 14 families from Europe, Asia and New Zealand have been reported. In an earlier study we have observed an increased risk of gastric cancer in families with hereditary prostate cancer. To evaluate the importance of possible germline mutations in the CDH1 gene in these prostate cancer families we screened exons 1-16 for mutations with the DHPLC technique. 24 samples from 18 families (15 families with hereditary prostate-gastric cancer and 3 with hereditary gastric cancer) were screened. We observed different patterns in Exons 1, 4, 5, 7, 10, 12, 13 and 15. For the samples with different patterns, a new PCR product was prepared, that was direct sequenced. In all samples but exon 10 we identified base-pair substitutions. In Exon 7 we identified a germ-line mutation, 1003C®T, that causes a stop-codon at the end of the exon in a family with hereditary diffuse gastric cancer. This mutation has previously been reported as a somatic mutation in a lobular breast tumour. In exons 1,4 and 13 we identified earlier reported polymorphisms. In exons 12 and 15 we identified 2 missense mutations with unknown significance in 2 families with hereditary prostate cancer. These mutations together with the mutation in intron 5 has not previously been reported as germline mutations. The prevalence of these three mutations were tested in 136 samples from 73 families with hereditary prostate cancer, 215 cases of sporadic prostate cancer and 422 age-matched controls using an allelic discrimination assay. None of these sequence variants were found in the extended material. In conclusion, we have identified a germline mutation in a family with hereditary gastric cancer and additional 9 sequence variants (2 missense, 6 polymorphisms in introns, 1 polymorphism in gene) of unknown significance. The three novel germline mutations found are pedigree specific and do probably not contribute to prostate cancer.

**Mutations in the BRCA1 and BRCA2 genes identified in a western Swedish patient cohort.** *J. Lundberg<sup>1</sup>, A. Bergman<sup>1</sup>, Z. Einbeigi<sup>2</sup>, Y. Engwall<sup>1</sup>, A. Flodin<sup>1</sup>, P. Karlsson<sup>2</sup>, T. Martinsson<sup>1</sup>, J. Wahlstrom<sup>1</sup>, A. Wallgren<sup>2</sup>, M. Nordling<sup>1</sup>.* 1) Dept. Clinical Genetics, Sahlgrenska Univ Hosp - East, Gothenburg, Sweden; 2) Dept. Oncology, Sahlgrenska Univ Hosp, Gothenburg, 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 mutation carriers among women with breast/ovarian cancer depends on the population studied, and displays 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 103 patients, selected under informed consent, from the Sahlgrenska University Hospital, Gothenburg, Sweden. The genes were initially screened using the Protein Truncation Test (PTT) on genomic DNA (BRCA1 exon 11, BRCA2 exon 10, 11) and cDNA from RT-PCR (all other exons) for truncating mutations. All mutations but one was detected with PTT; the remaining one with dHPLC. Automated DNA sequencing of the detected mutations revealed seven different frameshift mutations, two nonsense mutations and one large deletion. Four of these have not been reported earlier: BRCA1 409-410delCA; 2229-2230delAA; 3029delA; 1912 T>G. BRCA mutations were found in 32% of the screened families; this is comparable to frequencies reported in other European studies. Notably, a western Swedish founder mutation (BRCA1 3171ins5) accounted for 24 of the 33 mutations detected in the 103 families. Our results are furthermore in accordance with the observation that frameshift mutations in the first two-thirds of BRCA1 are associated with a higher risk of ovarian relative to breast cancer than are truncating mutations in the last one-third of the gene.



**Replication error and mismatch repair gene analyses of families with strong histories of colorectal cancer.** *K. Nafa, P. Peterlongo, E. Glogowski, J. Guillem, A. Markowitz, K. Offit, N.A. Ellis.* Memorial Sloan-Kettering Cancer Center, New York, NY.

Disease-causing mutations in the mismatch repair (MMR) genes MLH1 and MSH2 strongly predispose carriers to colorectal cancer and other cancers that have been associated with hereditary nonpolyposis colorectal cancer (HNPCC), as defined by the Amsterdam criteria. Such mutations have been associated with microsatellite instability (MSI) of the tumors that arise in these families. To define new genes that confer susceptibility to colorectal cancer, we have loosened the Amsterdam criteria to admit for study families with at least three first or second degree relatives affected with colorectal cancers diagnosed at any age. We have identified probands by questionnaires administered in clinics or by referral, and detailed family history information was obtained by patient interview. In nearly all of the 96 ascertained families from whom blood and/or tumor DNA was available, the occurrence of colorectal cancer was consistent with an autosomal dominant mode of inheritance. 49 of the families met Amsterdam criteria and the remaining 47 did not. So far, we have determined MSI status in 100 neoplasms (67 colon cancers, 26 adenomatous polyps, and 7 other cancers) from 95 persons in 64 of these families. In general, MSI status was concordant in families, with 23 families classed as MSI-high and 35 nonMSI-high. 6 families were discordant for MSI status. In 3 families, MMR gene mutations were detected by DNA sequencing; the discordance was explained by failure of MSI to develop i) in a polyp, ii) in a squamous cell carcinoma, and iii) in a polyp from a person who did not carry the familys mutation. In the remaining 3, an MMR gene mutation has yet to be found, and the MSI probably arose sporadically. 22 distinct mutations have been detected in 30 families (20 exhibited MSI, 1 did not, and 12 were not typed) with no mutation being found in 3 families with MSI-high tumors. Candidate gene analysis to find modifier genes in the mutation-positive families and linkage analysis to find novel genes in the nonMSI-high families is in progress.

**D-HPLC Detection of APC mutations.** *C.J. Meldrum, R. Crooks, R.J. Scott.* Molecular Genetics, Hunter Area Pathology Service, Newcastle, NSW, Australia.

The detection of mutations in the Adenomatous Polyposis Coli (APC) gene that is associated with Familial Adenomatous Polyposis (FAP) is both laborious and time consuming. Furthermore, the techniques used combined with limited resources dictates the extent to which mutation screening routinely proceeds. The effectiveness of a screening strategy utilising D-HPLC has been assessed in relation to the APC gene. Routine APC mutation screening in our laboratory consists of DGGE analysis for exons 1-14 combined with PTT of 4 overlapping fragments covering the 8.5 kb of exon 15. As an initial evaluation, D-HPLC has been used to screen exons 1-14 of the APC gene and to examine the regions of overlap inherent in the exon 15 PTT assay. The D-HPLC system detected all 10 mutations that had previously been identified in exons 1-14 by DGGE and confirmed by DNA sequencing. Half of these mutations were single base substitutions with the remaining five being a 1,2,4 and 14 base deletion and a 1 base insertion. Based on this number of mutations D-HPLC does not show a bias towards the detection of any particular type of mutation. Three mutations that had previously gone undetected by PTT due to their position within exon 15 were detected by D-HPLC analysis of the overlapping regions. D-HPLC analysis of 26 patients with histopathology strongly suggestive of FAP and a negative routine APC gene screening result is currently being used as an indication of the relative sensitivity of D-HPLC compared with DGGE and PTT for the detection of APC mutations. Initial results indicate that D-HPLC is at least as sensitive as DGGE and in one case detected a DNA change that was undetected by routine DGGE. Analysis of exon 15 by DHPLC has thus far revealed three missense mutations that would not be detected by PTT. The semi-automated nature of D-HPLC offers the ability to routinely analyse each PCR fragment of the APC gene under a number of conditions. This alone increases the mutation detection rate for this disorder. Sensitive and accurate detection of mutations in the APC gene is vital not only for diagnostic prediction of susceptibility to FAP but also for the identification of FAP phenotypic subgroups with a possible genetic cause other than an APC mutation.

**Identification of germline ATM mutations in breast cancer patients with normal BRCA1 and BRCA2 genes.** *M. Zhu<sup>1</sup>, N. Khanlou<sup>1</sup>, S.Y. Xue<sup>2</sup>, A. Diep<sup>2</sup>, V. Cortessis<sup>2</sup>, R. Haile<sup>2</sup>, R.A. Gatti<sup>1</sup>.* 1) Dept. of Pathology, UCLA School of Medicine, Los Angeles, California 90095-1732, USA; 2) USC Dept. of Preventive Medicine, Norris Comprehensive Cancer Center, Los Angeles, CA 90033-9175.

The genetic determinants for most breast cancer cases remain elusive; however, a mutation in a tumor suppressor gene, such as BRCA1, BRCA2, p53 or ATM (ataxia-telangiectasia mutated, ATM) has been suggested to be one mechanism of breast carcinogenesis. While germline mutations in BRCA1 and BRCA2 account for a significant proportion of hereditary breast cancer, their contribution to sporadic breast cancer is low. In such cases, ATM mutations may be an important risk factor. Evidence that ATM phosphorylates BRCA1 provides a possible mechanism by which ATM might participate in the development of breast cancer. We analyzed 52 samples from breast cancer patients who were BRCA1 and BRCA2 normal for mutations in ATM. Samples were screened by PCR-SSCP. SSCP variants were observed within 23 of the 66 ATM exons. Sequencing the corresponding fragments identified 10 mutations: 6 missense and 4 truncating mutations, 2 of the missense mutations had been previously observed. We also identified 36 variants, 30 of which were new. These results suggest that ATM mutations may play a role in the development of sporadic breast cancer.

**Colorectal and endometrial carcinomas from HNPCC and HNPCC-suspected patients show different MSI-profiles.** *Y. Wu<sup>1</sup>, M.J.W. Berends<sup>2</sup>, R.G.J. Mensink<sup>1</sup>, K.A. Kooi<sup>1</sup>, T. van der Sluis<sup>3</sup>, C. Kempinga<sup>3</sup>, R.H. Sijmons<sup>1</sup>, A.G.J. van der Zee<sup>4</sup>, H. Hollema<sup>3</sup>, J.H. Kleibeuker<sup>2</sup>, C.H.C.M. Buys<sup>1</sup>, R.M.W. Hofstra<sup>1</sup>.* 1) Dept. of Medical Genetics, Univ Groningen, Groningen, the Netherlands; 2) Dept. of Gastroenterology, Univ Groningen, Groningen, the Netherlands; 3) Dept. of Pathology, Univ Groningen, Groningen, the Netherlands; 4) Dept. of Gynaecology, Univ Groningen, Groningen, the Netherlands.

Due to defective mismatch repair, tumors of HNPCC patients often show microsatellite instability (MSI). We determined the frequency of MSI in colorectal (CRC) and endometrial carcinomas (EC) from HNPCC and HNPCC-suspected patients and evaluated the use of a consensus panel of microsatellite markers (BAT25, BAT26, BAT40, D2S123, D5S346 and D17S250). MSI analysis was performed in tumors from 37 unrelated HNPCC patients and in tumors from 314 patients suspected for HNPCC. 89% (23/26) of the CRC and 82% (9/11) of EC tumors of HNPCC patients showed MSI. Tumors from patients suspected for HNPCC showed MSI in 35% (83/239) of CRC and 30% (26/88) amongst EC. Comparing the performance of the different markers in the different tumor types we found that mononucleotide repeats were much less informative in EC than in CRC. Colorectal carcinomas from HNPCC patients showed 76%-82% MSI with mononucleotide repeat markers and 73%-78% with dinucleotide markers. For HNPCC-suspected patients these figures were 44%-64% and 63%-84%, respectively. Endometrial carcinomas from HNPCC patients showed 11%-44% MSI with mononucleotide repeat markers and 56%-78% with dinucleotide repeat markers. For HNPCC-suspected patients these figures were 27%-44% and 58%-72%, respectively. Our results show that a substantial proportion of CRC and EC from HNPCC-suspected patients has an MSI-H tumor phenotype and that the different types of markers have different sensitivities for colorectal cancers and endometrial carcinomas. This implies that for MSI analysis in endometrial cancer and maybe other extracolonic cancers (a) different set(s) of consensus markers may be needed.

**An early-onset breast and colorectal cancer-prone family: Does a specific hereditary breast and colorectal cancer syndrome exist?** *I. Thiffault<sup>1</sup>, T. Pal<sup>5</sup>, N. Hamel<sup>4</sup>, J. Deschênes<sup>3</sup>, V. Marcus<sup>3</sup>, F. Odefrey<sup>5</sup>, K. Watters<sup>3</sup>, T. Graham<sup>7</sup>, W. Meschino<sup>7</sup>, S. Narod<sup>6</sup>, D. Goldgar<sup>5</sup>, D. Farber<sup>1</sup>, E. MacNamara<sup>1,2</sup>, G. Chong<sup>1,2</sup>, W. Foulkes<sup>1,2,4</sup>.* 1) SMBD-Jewish General Hospital; 2) Dept. of Medicine; 3) Dept. of Pathology; 4) RIMUHC, McGill University, Montreal, QC, Canada; 5) IARC, Lyon, France; 6) Women's College Hospital; 7) Sunnybrook Regional Cancer Centre, Toronto, ON, Canada.

We describe a large pedigree with 15 1st-, 2nd- or 3rd-degree relatives who have developed colorectal cancer (CRC) (n=4, 3 <45 yrs old); breast cancer (BC) (n=5, all <45 yrs); endometrial cancer (n=2); lung cancer (n=2), ovarian cancer, anal cancer, multiple myeloma, astrocytoma and thyroid cancer (all n=1). Linkage analysis suggested a mutation was present in BRCA2 and direct sequencing of DNA from a woman with lobular breast cancer identified a L105X mutation in BRCA2. Analysis of a 6 mm colonic villotubular polyp from her sister who had had both invasive breast and endometrial cancer with BAT26 suggested MSI. RNA analysis by PTT and cDNA sequencing revealed a deletion of MSH2 exon 8 in this patient. This case is the first reported incidence of double heterozygote for truncating mutations in BRCA2 and MSH2. Linkage and mutation analyses in this family revealed that her father is an obligate double heterozygote. Three individuals carry the MSH2 but not the BRCA2 mutation. Six other individuals carry the BRCA2 mutation, but not the MSH2 mutation. 4/4 BRCA2-related breast cancers were ER+. There has been interest in the possible existence of a gene that predisposes to both BC and CRC. In addition, the risk of BC in HNPCC families, and the risk of CRC in BRCA1/BRCA2 families has been in dispute. Our identification of a mutation in one mismatch repair (MMR) gene and one breast cancer susceptibility gene, with no evidence that women in this pedigree were susceptible to BC solely because they carried a MMR gene mutation, strikes a note of caution both to those looking for a BC-CRC gene and those who consider BC to be part of HNPCC. We suggest that BC and CRC in families with several cases of early-onset BC, in a setting of HNPCC, may be attributable to more than one gene.

**Analysis of MSH6 mutations by denaturing high performance liquid chromatography (DHPLC) in HNPCC families.** *P. Peterlongo<sup>1</sup>, K. Nafa<sup>1</sup>, G. Lerman<sup>1</sup>, S. Pawar<sup>2</sup>, J. Guillem<sup>1</sup>, A. Markowitz<sup>1</sup>, K. Offit<sup>1</sup>, N.A. Ellis<sup>1</sup>.* 1) Memorial Sloan-Kettering Cancer Center, New York, N.Y; 2) IMPATH, New York, N.Y.

Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common form of hereditary predisposition to colorectal cancer. It is primarily caused by germline mutations in the mismatch repair (MMR) genes MLH1 and MSH2 and is associated with a tumor phenotype featuring instability at microsatellite repeat sequences that is referred to as the replication error (RER). Several recent studies have shown mutations in MSH6 can cause HNPCC; however, some MSH6-mutated tumors are RER-positive and some are not. In this study, we used DHPLC followed by direct sequencing of the variant fragments to screen for mutations in MSH6 in a collection of 41 HNPCC families fulfilling Amsterdam or HNPCC-like criteria and in which MMR gene mutations had not been found. The tumors in 13 of these families were RER-positive and in 28 they were not. To validate the method, genomic DNA samples from 11 additional unrelated, affected persons from RER-untyped HNPCC families were screened by both DHPLC and direct sequencing for mutations in MSH6. In 42 fragments with an abnormal profile by DHPLC a total of 12 heterozygous DNA polymorphisms were detected. All heterozygous sequence variants found by direct sequencing were also detected by DHPLC. In the 41 RER-typed HNPCC families, we found 16 known non-pathogenic polymorphisms. Not reported previously was a 39G to S missense mutation found in a person with an RER-negative tumor. One pathogenic MSH6 deletion was found, 3511delGA, that gives an immediate stop codon in exon 6 (1171A to X) in a family that fulfills the Amsterdam criteria; the probands tumor was RER-positive. This family presents 6 cases of colorectal cancer and 1 uterine cancer involving 3 generations (mean age of onset 42). Our experience suggests that mutation in MSH6 is responsible for a small proportion of HNPCC families.

**Measurement of mutation load using ethanol-fixed, paraffin-embedded and immunohistochemically-stained human tissues.** *G. Schlake, K.A. Hill, E. Heinmoeller, J. Chen, X. Li, Q. Liu, J. Still, S.S. Sommer.* Molecular Genetics, City of Hope Ntl Med Ctr, Duarte, CA.

Individuals who have a high mutation load in their normal tissues are presumably at increased risk for cancer. This presumption has not been tested because it has not been possible to measure mutation load (mutation frequency and alterations in mutation pattern and spectrum) in vivo using an endogenous human gene. We have developed a method for measuring mutation load from ethanol-fixed, paraffin-embedded human tissues immunohistochemically-stained with anti-p53 antibodies. The p53 gene is used because many missense changes in exons 5 to 9 cause accumulation of the protein. Speculation that the p53 gene is the guardian of the genome notwithstanding, compelling data indicate that the frequency and pattern of transitions, transversions, and microdeletions are unchanged in normal tissues from p53 nullizygous mice that develop cancer early. This suggests that the p53 gene is a good mutation reporter because the background rate of mutations in cells lacking functional p53 is similar to that of wild type cells. In this mutation load assay, single cells that stain positively for a mutant reporter gene such as p53 are microdissected. Genomic DNA is amplified using a novel PCR protocol specifically suited to amplify large amounts of a long (2 kb) template from single cells. The method is generally applicable to analysis of mutations from single cells in tissue sections. Somatic mutations have been identified from single cells from colon, liver, lung, breast and kidney. We have also developed the means to reduce the rate of allele dropout and polymerase error, crucial to estimates of mutation load. This method permits comparison of normal tissues and cancers and identification of individuals at risk for cancer. Individuals with putative exposure to environmental mutagens may be analyzed as well as individuals with possible DNA repair defects and a history of familial cancer.

**The nature of the stimulus affects the control of the dominant negative effect of p53 mutants.** *W. Dridi<sup>1</sup>, R. Feti<sup>2</sup>, J. Lavoie<sup>1</sup>, MF. Poupon<sup>3</sup>, R. Drouin<sup>1</sup>.* 1) Medical Biol, Hosp St Francois, Laval Univ, Quebec, Quebec, Canada; 2) Hematologie Dept, Hosp Maisonneuve-Rosemont, Montreal, Quebec, Canada; 3) Cytogenetique Moleculaire et Oncologie, Institut Curie, Paris, France.

P53 mutant proteins possess a dominant negative activity, which is under the control of several factors namely the p53 mutation and the cellular environment. In our study, we examined the cellular response of the near-diploid LoVo colon carcinoma cell line possessing two wild-type p53 alleles (wild-type clone) and three other clones transfected with different p53 mutants (273his, 173his and 143ala) to different spindle inhibitor (nocodazole and Colcemid) treatments. HCT116 cell lines, which are wild-type for the p21 allele, and three other isogenic cell lines (80p21+/-, 80S4p21-/- and 80S14p21-/-) were also studied. Flow cytometric studies, analysis of retinoblastoma protein (pRB) dephosphorylation and 5-bromo-2-deoxyuridine (BrdU) incorporation by immunocytochemistry revealed a tetraploid G1 arrest of the wild-type LoVo clone as well as the 273his mutant clone after exposure to nocodazole, preventing tetraploid cells from entering in an additional S phase. Whereas the 173his and 143ala mutant clones re-enter S phase. On the other hand, it has been shown that when the cells are exposed to ionizing radiations, the wild-type LoVo and the 143ala mutant clones possess a diploid G1 arrest, whereas the 273his and 173his mutant clones re-enter S phase. Therefore, our results suggest that p53 mutant dominant negative activity is dependent not only on cellular environment and the type of p53 mutation, but also on the nature of the stimulus.



**The analysis of LOH target genes in 11q21-q24.** *M. Allinen<sup>1</sup>, L. Peri<sup>1</sup>, K. Outila<sup>1</sup>, S. Kujala<sup>1</sup>, J. Lahti-Domenici<sup>1</sup>, V. Launonen<sup>2</sup>, R. Winqvist<sup>1</sup>.* 1) Department of Clinical Genetics, University of Oulu/Oulu University Hospital, Oulu, Finland; 2) Department of Medical Genetics, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland.

Loss of heterozygosity (LOH) screening has widely been used to identify locations of tumor suppressor genes. LOH at the distal half of chromosome 11q has frequently been seen in a variety of human tumors including breast cancer, and is often associated with worse prognosis. In the ongoing attempt to locate and characterize the target genes contained in the indicated chromosome region, we have first looked for aberrations in known genes indicated to be involved in tumorigenesis. We have examined 32 primary breast tumors showing LOH in 11q21-q24 for mutations in the ATM, PPP2R1B, MRE11A, TSLC1 and CHK1 genes, all of which link to pathways related to cancer development, or have been shown to suppress tumor formation. Only in one of the analyzed tumors a putative pathogenic alteration occurring in PPP2R1B was detected. No other genetic changes were seen.

The absence of intragenic mutations made us next evaluate the possibility of gene silencing by promoter region CpG hypermethylation by using the bisulfite sequencing technique. We found the ATM, PPP2R1B, MRE11A and CHK1 promoter regions mostly unmethylated, excluding some occasional residues. For TSLC1, three tumors showed methylation of the six analyzed CpG sites. However, at most the methylation occurred in 45% of the analyzed clones. Whether the hypermethylation of the TSLC1 promoter region really represents the 'second hit' along with LOH in breast cancer needs to be confirmed in more extensive studies.

**Variation of DNA sequences encoding the polymorphic polyglutamine tract of AIB1.** *P. Dai, L.J.C. Wong.* Inst Molec & Human Genetics, Georgetown Univ Medical Ctr, Washington, DC.

Amplified in breast cancer1 (AIB1) gene is a member of the steroid receptor coactivator (SRC-1) family. The gene was discovered and cloned on the long arm of chromosome 20 that was over-expressed in human breast cancers. AIB1 gene contains a polymorphic polyglutamine(polyQ) tract toward the C terminal. Recent Reports have shown conflict results on the association of shorter polyQ repeat with an increase risk of breast cancer. Since the polyQ tract was not encoded by perfect CAG repeats, the interruption by CAA codon may play a role in AIB1 gene amplification and its effect on tumorigenesis. The aim of this study is to examine the DNA sequences encoding the polyQ tract of AIB1 gene and to study its relationship with breast cancer. Due to the polymorphic heterozygous feature of the CAG repeat region, direct sequencing was not possible to clearly resolve the sequence of the two heterozygous alleles. We, thus, PCR amplified the region containing CAG repeat, and subsequently cloned these fragments into pCR2.1-TOPO vector, followed by DNA sequencing. Multiple clones(>6) from each of more than 62 DNA samples including breast cancer cell lines, tumor tissues, blood samples from patients with germline mutations in brca1/brca2, and healthy controls were sequenced. At least 13 different triplet repeat coding patterns were found. Two of these sequences were considered common because of their high frequency in both normal and cancer groups. The remaining were rare sequence patterns and most of them occurred only once or twice. The results of statistical analysis showed significant higher proportion of breast tumor or cancer cell line specimens are associated with rare sequence patterns and shorter alleles when compared to healthy individuals. Real time quantitative PCR analysis of AIB1 gene revealed gene amplification in several breast tumor cell lines and at least 1 tumor sample but not in normal controls. Our results suggest that both qualitative and quantitative difference in the polyQ encoding region may contribute to the role of AIB1 gene in the tumorigenesis of breast cancer.

**Loss of Tyrosine Aminotransferase may Contribute to the Pathogenesis of Hepatocellular Carcinoma.** *X.Y. Guan, Y.W. Xie, J.S.T. Sham.* Dept Clinical Oncology, Univ Hong Kong, Hong Kong, China.

Loss of material at chromosome arm 16q is one of the most frequent alterations in hepatocellular carcinoma (HCC) detected by comparative genomic hybridization and loss of heterozygosity studies. It suggests that the frequent deleted region at 16q may contain a tumor suppressor gene. cDNA subtraction strategy was applied to isolate down-regulated gene at 16q. cDNAs from the surrounding non-tumor liver tissue was subtracted with cDNAs from its matched primary HCC tumor and a gene at 16q22, tyrosine aminotransferase gene (TAT), was isolated. RNA expression of the TAT gene in 8 primary HCC tumors with 16q deletion was analyzed by northern blot hybridization and reverse transcriptase-polymerase chain reaction. Absence and marked reduction expression of TAT gene was detected in 5 and 2 tumors, respectively, as compared with tumor surrounding liver tissues. Homo-deletion of genomic DNA sequence of TAT gene was observed in two cases detected by PCR. Tyrosine aminotransferase is the rate-limiting enzyme for the tyrosine catabolic pathway and the deficiency of TAT gene may result in elevation of tyrosine level. Prolonged elevation of intracellular tyrosine concentrations may disrupt normal functions of hepatocytes and initiate local inflammatory responses, leading to liver diseases including HCC. Therefore, loss of TAT gene may contribute to the pathogenesis of HCC.

**Amplification and Overexpression of Multiple 17q Genes in Breast Cancer.** *A.E. ERSON*<sup>1</sup>, *B.L. NIELL*<sup>2</sup>, *E.M. PETTY*<sup>2</sup>. 1) Department of Human Genetics,; 2) Department of Internal Medicine, Division of Medical Genetics, University of Michigan, Ann Arbor, MI 48109.

Gene amplification is a common mechanism through which proto-oncogenes are overexpressed and contribute to tumor progression. Comparative genomic hybridization (CGH) and fluorescent in situ hybridization (FISH) studies have revealed several regions of amplification on chromosome 17q in breast cancer. One well-known amplicon on 17q21 is associated with overexpression of the ERBB2 (HER-2/NEU) proto-oncogene. In search of novel proto-oncogenes on 17q, we screened genes and ESTs in physical and radiation hybrid maps for amplification and overexpression in 24 breast cancer cell lines by semi-quantitative duplex PCR, semi-quantitative duplex RT-PCR, Southern and Northern blot analyses. We identified two distinct amplicons on 17q23, one including TBX2 and another proximal region including RPS6KB1 and MUL. 17q23 is of particular interest since it is a gene rich region and amplification of 17q23 has been frequently described in breast cancer. Amplification and overexpression of 17q23 genes (RAD51C, RPS6KB1 (PS6K), NACA, PAT1, SIGMA1B and TBX2) have been reported in breast cancer. We also identified significant overexpression of USP6 (TRE2 oncogene) in 2 breast cancer cell lines. USP6 is a ubiquitin specific protease and maps to 17q11. In conclusion, different regions of 17q are amplified and overexpressed in breast cancer. Further functional studies will reveal the roles of these overexpressed genes in breast tumorigenesis.

**Genetic polymorphism of CYP2D6 and lung cancer risk.** *L. Arnaud<sup>1</sup>, M.P. Gallegos<sup>1</sup>, B.C. Gomez<sup>1</sup>, G. Morgan<sup>2</sup>, M.C. Flores<sup>1</sup>, H. Rangel<sup>3</sup>, G.M. Zuiga<sup>1</sup>.* 1) Medicina Molecular, CIBO, IMSS, Guadalajara, Jalisco, Mexico; 2) Division de Oncologia, Hospital de Especialidades, CMNO, IMSS, Guadalajara, Jal. Mexico; 3) Departamento de Ciencias Basicas, Laboratorio de Genetica Molecular, Centro Universitario de Ciencias Basicas, Universidad de Guadalajara.

Previous reports of the association of debrisoquine metabolism, controlled by the cytochrome P450 CYP2D6, with increased lung cancer risk have been conflicting. We examined the hypothesis that genetic polymorphism at the CYP2D6 locus identifies individuals at increased risk for lung cancer in case-control study of 53 incident mexican lung cancer patients and 152 age, and matched controls. Using polymerase chain reaction-RFLP to detect CYP2D6 (G1943A, PM), wich revealed the presence of 18.86% poor metabolizer in the patients and 5.04% in the controls. A statistical diffence was found to compared the previous data. Our data suggest that the CYP2D6 (G1943, PM)genetic polymorphism increased 3.5 fold the risk for mexican lung cancer.

**Analysis of consequences of WT1 splicing alterations on transcription regulation in Wilms' tumors using cDNA expression arrays.** *D. Baudry, M. Rigolet, M. Hamelin, M. Faussillon, M.O. Cabanis, C. Junien, C. Jeanpierre.*  
INSERM UR383 , Hopital Necker Enfants-Malades, Paris, France.

Somatic WT1 mutations are rare in sporadic Wilms' tumors, although constitutional mutations of this gene are clearly associated with predisposition. It has been suggested that abnormal splicing may be another mode of somatic WT1 alteration. To investigate in detail WT1 changes at the somatic level, we recently analyzed the levels of the four isoform transcripts produced by alternative splicing events in a series of 50 tumors. We characterized splicing alterations in 63% of sporadic WT. Moreover, taking into account the decreased and increased overall levels of WT1 mRNA, the percentage of sporadic tumors with changes in WT1 expression reached 90% .

To investigate the relationship between the variation of the isoform ratios and the tumorigenic process, we compared the expression profiles of tumors with different isoform ratios, using a macroarray approach. Because of Wilms' tumors heterogeneity, we chose to use pools of samples. We hybridized Atlas Human Cancer cDNAs expression arrays (Clontech) with cDNA from pools of (1) 5 tumors with normal exon 5 +/- isoform ratio (T4); (2) 5 tumors with abnormal exon 5 +/- isoform ratio (T1); (3) 4 normal kidneys (NK) and (4) 5 fetal kidneys (FK). We thus identified 15 genes which present a specific expression profile in T1 compared to T4, NK and FK. We validated the approach by analysing the expression level of 4 of the 15 genes in samples taken individually and pooled by RT-PCR. Furthermore, we analyzed one candidate gene by real-time RT-PCR method in a series of 50 tumors and ascertained its differential expression in correlation with alternative splicing of WT1. By this approach, we are expecting to identify not only new WT1 targets but also the potentially alternative pathway(s) involved in Wilms' tumorigenesis.

**Suppressor of fused (SUFU), a conserved negative regulator of the hedgehog (HH) pathway, reverses the tumorigenic phenotype of a rhabdomyosarcoma cell line.** *A.F. Cooper, A.E. Bale.* Yale University School of Medicine, New Haven, CT.

Elucidating the molecular pathogenesis of cancer may allow for the development of pharmacological agents that modulate relevant pathways and set the stage for rational medical therapy. The HH signaling pathway plays a key role in embryogenesis and also regulates cell growth and differentiation in the adult. Mutations of patched (PTCH), the HH receptor, render this pathway constitutively active in almost all basal cell carcinomas, a substantial fraction of medulloblastomas, and occasionally in rhabdomyosarcomas and breast cancers. One strategy for counteracting the effects of PTCH mutations would be to activate a downstream gene that negatively regulates the pathway. Studies in *Drosophila* suggest that *sufu* may play this role in fruit flies. To test the ability of human SUFU to suppress neoplastic characteristics of tumor cells, we generated tetracycline repressible stable transfectants in the rhabdomyosarcoma cell line, TE671. TE671 has moderate levels of expression of HH target genes, suggesting some activation of the HH pathway, and very low levels of endogenous SUFU expression. To test the effects of SUFU overexpression we employed standard tissue culture-based assays that correlate with the ability of cell lines to form tumors *in vivo*. A TE671 line bearing the tetracycline repressible system but lacking a functional copy of SUFU was used as a negative control. Induction of SUFU caused a minor decrease in colony forming ability and in growth rate as assessed by thymidine incorporation assays. In contrast to the small effect on cell kinetics, there was a distinct morphologic change in which the cells became elongated and spindle shaped, consistent with myogenic differentiation. This morphologic alteration was accompanied by complete loss of anchorage-independent growth in a soft agar assay. The change in biological behavior was associated with a substantial alteration of expression of TGF $\beta$ 1, an HH pathway target gene. Evaluation of other target genes is in progress. These data show that SUFU has a specific and powerful effect on differentiation and anchorage independent growth that is probably caused by modulation of the HH pathway.

**Evaluation of candidate genes from the chromosome 20q13.2 amplified region in breast cancers.** *L. Anderson<sup>1</sup>, C. Ginther<sup>1</sup>, M. Schoenberg-Fejzo<sup>1</sup>, K. Anders<sup>2</sup>, D.J. Slamon<sup>1</sup>.* 1) Department of Medicine, University of California, Los Angeles, CA; 2) Department of Pathology, Southern California Permanente Medical Group, Woodland Hills, CA.

Comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) studies have demonstrated the existence of three distinct amplified regions on chromosome 20q in breast cancers, at 20q11, 20q12, and 20q13.2. We sought to evaluate several potential candidate target genes of the 20q13.2 amplified region in a series of primary breast malignancies for which matched DNA and RNA were available. We tested candidate genes ZNF217, CYP24, AURORA 2 and TFAP2C for amplification and overexpression on Southern and Northern blots in 24 primary breast cancers as well as in breast and lung cancer cell lines. The published order of these genes on chromosome 20q13.2 is Centromere--- ZNF217---CYP24---AURORA 2---TFAP2C---Telomere. Two of 24 samples (8%) showed high level (>5X) amplification for ZNF217. The amplification was matched by high level mRNA expression of ZNF217. AURORA 2 was also amplified and overexpressed in the same two samples, although at a lower (2-5X) level. CYP24 was amplified and overexpressed at a low level (2-5X) in one of the two samples. Neither sample demonstrated high or low level amplification or overexpression of TFAP2C. We did not detect amplification in any of the other 22 primary breast cancers with the candidate genes. However, AURORA 2, CYP24 and TFAP2C, but not ZNF217, had increased mRNA expression in the absence of amplification in several primary cancers. Our study supports the concept that ZNF217 is at least one of the candidate targets of the 20q13.2 amplicon in breast cancers. In addition, AURORA 2 and CYP24 cannot be ruled out as contributing to the cancer phenotype. TFAP2C does not appear to be a target gene in these samples.



**Differential Gene Expression in Malignant Breast and Colon Cancer Cells and their Suppressed Counterparts.**

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The majority of genes responsible for various aspects of malignancy have yet to be identified for their roles as such. To identify some of these genes, we have analyzed gene expression profiles in a pair of matched cell lines which differ greatly in their phenotypic behavior. Three hybrid cell lines were previously developed via MMCT of a normal copy of chr 8, which has been implicated as harboring a tumor suppressor gene, into two breast (MB231 and ZR75) and one colon (HT29) cancer cell lines. These hybrid cell lines differ from the parental cells by demonstrating suppression of some aspect of malignancy. The hybrid cells, HT29.X8 and MB231.X8, showed suppression of growth in soft agar and tumorigenicity in nude mice, while ZR75.X8 showed suppression of only clonicity. We performed comparative gene expression analysis of the HT29 cell line pair using GeneFilters arrays (Research Genetics). Only a small subset of genes was consistently altered upon introduction of chromosome 8. These genes were selected on the basis of (1) showing > 2-fold increase, and (2) variability of expression levels between repeat experiments less than 40%. Four genes met these criteria: RNF11, CED-6, KIAA0663, and PMEPA1. Semi-quantitative RT-PCR analysis confirmed that two genes (RNF11 and KIAA066) demonstrated 1.5-2 fold increase of expression from parent to hybrid, CED-6 showed a 2.5 fold increase, and PMEPA1 was increased in the hybrid by over 20-fold. In the breast cancer cell lines, PMEPA1 showed an increase of 45 fold in the chromosome 8-suppressed ZR75 cells, and KIAA0663 showed an increase of 2.6 fold in the chromosome 8 - suppressed MB231 cells. Studies evaluating the functional significance of these genes and their relevance to carcinogenesis are currently underway.

**Introduction of chromosome 18 suppresses the growth of the human osteosarcoma cell lines OHS50. T.L.**

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Loss of heterozygosity (LOH) studies have implicated chromosome arm 18q as the site for a tumor suppressor gene important in osteosarcoma tumorigenesis. Previously, LOH studies were performed on sporadic osteosarcomas and Pagetic osteosarcomas to localize the region of LOH on chromosome 18q. Using mitotic mapping, a minimal region of LOH was defined between markers D18S60 and D18S42 on chromosome 18q. This region is lost in 65-70% of osteosarcoma tumors, which implicates this region as the site of a tumor suppressor gene. In order to develop a functional assay for this tumor suppressor gene, microcell-mediated chromosome transfer was used to introduce an intact copy of chromosome 18 into three independent osteosarcoma cell lines: SAOS-2, OHS50, and U2 OS.

From all three fusion experiments, numerous microcell hybrid clones, containing an intact chromosome 18, were analyzed by *in vitro* methods for transformed phenotypes, including analyses of doubling time and ability to grow in soft agar. Introduction of an intact chromosome 18 into the SAOS-2 and U2 OS cell lines did not cause any significant changes in *in vitro* growth parameters. However, microcell hybrids isolated from the introduction of chromosome 18 into OHS50 cells exhibited dramatic changes in the doubling time and ability to grow in soft agar. The doubling time was increased 2.1-fold compared to the OHS50 control and the ability to grow in soft agar was reduced 4-fold.

OHS50 is an ideal model system for the identification of a tumor suppressor gene on 18q involved in osteosarcoma tumorigenesis. Future experiments will explore the *in vivo* phenotype of these hybrids.

**Familial aggregation of non-melanoma skin cancer.** *G.J. Mann<sup>1</sup>, C.H-F. Thoo<sup>1</sup>, L. Morgan<sup>1</sup>, M. Stewart<sup>2</sup>.* 1) Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute Westmead, NSW, Australia; 2) Skin and Cancer Foundation, Australia.

Cutaneous squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) are Australia's most prevalent and costly cancers, together affecting over 20% of the population at some time in their life. SCC accounts for almost one third of all non-melanoma skin cancer (NMSC), and is responsible for most of the deaths due to NMSC. However SCC is one of the few common malignancies for which major susceptibility genes are yet to be identified. We are therefore analysing familial aggregation of cancer in individuals diagnosed with early onset SCC. Family histories have been verified for first degree relatives of 28 probands under the age of 50, identified so far in Sydney from the referral pathology database of the Skin & Cancer Foundation, Australia. Among siblings (n=70), 10.0% had developed NMSC compared with 1.6% expected from national survey data. Among parents (n=36), 33.3% had developed NMSC compared with 13.4% expected. Excesses of both SCC (RR 5.7) and BCC (RR 2.7) were observed in these relatives, but are significant ( $p < 0.05$ ) only for SCC as yet. Overall, 46% of probands had a positive family history of NMSC and 14% belonged to clusters of three or more cases related in the first degree. These results confirm that NMSC exhibits significant familial aggregation and suggest that at least some of this familial risk is due to SCC-specific factors. Recruitment and extension of such kindreds will yield a useful resource for determining which genes are responsible.

**Tamoxifen and breast cancer incidence among women with inherited mutations in BRCA1 and BRCA2: A genomic resequencing study in the NSABP-P1 Breast Cancer Prevention Trial (BCPT).** *M.C. King<sup>1</sup>, H.S. Wieand<sup>2</sup>, K. Hale<sup>1</sup>, T. Walsh<sup>1</sup>, K.M. Owens<sup>1</sup>, M.K. Lee<sup>1</sup>, J.P. Costantino<sup>2</sup>, B. Fisher<sup>2</sup>, NSABP Investigators<sup>2</sup>.* 1) Depts of Medicine and Genomic Sciences, Univ Washington, Seattle, WA; 2) NSABP, Univ Pittsburgh, Pittsburgh, PA.

The randomized, double-blind BCPT demonstrated that tamoxifen reduced incidence of invasive BC by about 50% in high risk women. Specifically, tamoxifen reduced incidence of estrogen receptor (ER) positive BC: for ER positive BC, the tamoxifen-placebo risk ratio RR=0.31, 95% CI (0.22, 0.45). Tamoxifen had no effect on incidence of ER negative BC: RR=1.23, 95% CI (0.74, 2.03). In order to evaluate whether tamoxifen reduced BC incidence among women with inherited mutations in BRCA1 or BRCA2, we sequenced BRCA1 and BRCA2 (24,133 bp per sample) for women who developed invasive BC during the BCPT and for a stratified random sample of participants who remained unaffected. In all, 18.5 Mbp were evaluated. Of the 315 women who developed BC during the BCPT, DNA was obtainable and BRCA1 and BRCA2 sequenced for 288 (91%). Of these, 19 (6.6%) carried inherited, disease-predisposing mutations in BRCA1 or BRCA2: 17% of those diagnosed < 50 years and 3% of those diagnosed at age 50+. Of eight participants with BRCA1 mutations who developed BC, five were assigned to tamoxifen and three to placebo: RR=1.67, 95% CI (0.41, 8.00). Of 11 women with BRCA2 mutations who developed BC, three were assigned to tamoxifen and eight to placebo: RR=0.38, 95% CI (0.06, 1.56). For the 269 wildtype cases, RR=0.48, 95% CI (0.37, 0.61). More than 80% of BRCA1 breast tumors are ER negative, whereas ~80% BRCA2 breast tumors are ER positive. Hence, no effect of tamoxifen on BC incidence among women with BRCA1 mutations is consistent with no effect of tamoxifen on incidence of ER negative BC. In contrast, the (not significant) 62% reduction in BC incidence among women with BRCA2 mutations is consistent with the 69% reduction in incidence of ER positive BC. This study addressed incidence of new BC, not treatment by tamoxifen of existing BC. For women with ER positive BC, regardless of genotype, tamoxifen is effective in reducing risk of recurrence of disease.

**The EMX2 homeobox gene suppresses growth of the HEC-1A endometrial cancer cell line.** *F.C. Noonan<sup>1</sup>, C.R. Todd<sup>1</sup>, D.G. Mutch<sup>2</sup>, P.J. Goodfellow<sup>1</sup>.* 1) Dept Surgery, Washington Univ, St Louis, MO; 2) Dept OB/GYN, Washington Univ, St Louis, MO.

Our laboratory identified EMX2 as a candidate for the 10q15.3-q26.1 endometrial cancer tumor suppressor gene. EMX2 has known roles in embryonic development. We showed that the gene is also expressed in adult tissues, specifically in the endometrium (Noonan et al., 2001). Expression analysis in normal endometrium, tumors and endometrial cancer cell lines revealed EMX2 is expressed at higher levels in the quiescent post-menopausal endometrium than in the proliferating endometrium, consistent with a role in growth suppression. Primary endometrial cancers frequently show reduced levels of EMX2 and 4 of 6 endometrial cancer cell lines investigated do not express EMX2. Mutation analysis in primary tumors revealed a low frequency of mutations and the absence of detectable mutations in endometrial cancer cell lines (Noonan et al., 2001). Methylation studies show that 3 of the 4 cell lines that do not express EMX2 have extensive promoter methylation, suggesting that epigenetic silencing of EMX2 may be responsible for the absence of expression in the cell lines.

To further characterize the role EMX2 plays in endometrial tumorigenesis, we have performed two functional assays using HEC-1A cells (an endometrial cancer cell line that does not express EMX2) transfected with a pcDNA expression vector with or without the EMX2 gene: doubling time and clonogenic assays. We show here that cell lines that stably express the EMX2 transgene grow slower than cells that do not express EMX2. The difference in mean doubling times (76 hours for expressing lines and 46 hours for non-expressing lines) is statistically significant ( $p=0.0002$ ) and supports EMX2's role in growth suppression. Additionally, clonogenic assays with pcDNA and other expression vectors show similar results. HEC-1A cells transfected with EMX2-expression vectors form fewer colonies than cells transfected with an empty vector. A detailed functional characterization of EMX2 will help to elucidate the function of this gene in adult development and cancer progression.

**Identification of Breast Cancer Progression Genes Using Microarray Analysis.** *M.A. Unger<sup>1,2</sup>, W.F. Foster<sup>6</sup>, R. Huber<sup>6</sup>, M. Rishi<sup>5</sup>, E.A. Keiper<sup>3, 4</sup>, L.A. Chodosh<sup>3,4</sup>, M.N. Liebman<sup>2</sup>, B.L. Weber<sup>1,2</sup>.* 1) Univ. of Penn Cancer Center; 2) Abramson Family Cancer Res. Inst; 3) Dept. of Mol. and Cell Engin; 4) Div. of Endo., Diab., and Met., Univ. of Penn School of Med., Phila., PA; 5) Dept. of Path., St. Francis Hospital, Wilm., DE; 6) Applied Biotech., Dupont Pharm., Wilm., DE.

The current model for breast cancer progression describes a multistage process where normal breast epithelium is transformed to a carcinoma in situ due to the accumulation of genetic alterations, followed by a additional genetic changes that result in the transition to an invasive carcinoma and eventually metastasis. This study was designed to identify genes whose expression is altered between normal breast tissue and invasive ductal carcinoma tissue from the same individual. Fresh frozen tissue was collected and macrodissected with a pathologist to identify populations of cells that were 85% or greater homogeneous normal breast tissue or invasive carcinoma. To date, five matched tumors and normal pairs have been examined using oligonucleotide microarrays. Data analysis using Rosetta Resolver software pinpoint approximately 150probe sets representing transcripts consistently expressed at significantly lower levels in the tumors compared to the normal tissue from the same person and 50 transcripts expressed at significantly higher levels in the tumor compared to the normal. Approximately 20% of these transcripts are known markers of cancer. The 20 upregulated genes with the most significance changes reveal that 20% of these genes are histone genes and 15% are mucin genes, both of which have known implications in cancer progression. Of the down-regulated genes, 15% were genes that encode tight junction proteins, 15% were genes involved in immune surveillance, and 10% were genes with known cell cycle suppression functions. Additional data analysis suggests a clear involvement of a variety of S100 proteins and homeobox proteins, among others, in the transition of normal to invasive carcinoma as well as the contribution of a variety of genes that have not been previously implicated in breast cancer progression.

**g-synuclein, a candidate oncogene, activates RAC and ERK and contributes to the metastatic spread of breast and ovarian cancer.** Z. Pan<sup>1</sup>, W. Bruening<sup>1</sup>, B.I. Giasson<sup>2</sup>, V.M. Lee<sup>2</sup>, A.K. Godwin<sup>1</sup>. 1) Fox Chase Cancer Center, Philadelphia, PA; 2) U. of Penn. School of Medicine.

The synucleins (a, b, g, synoretin) are a family of small cytoplasmic proteins that are predominantly expressed in neurons. Although a-synuclein is known to play an important role in neurodegenerative diseases, the function of synucleins is unknown. We have previously reported that one member of the family, g-synuclein, is expressed in the majority (>85%) of late-stage breast and ovarian carcinomas, but it is not expressed in normal mammary and ovarian epithelium. Therefore, we hypothesize that g-synuclein may be a proto-oncogene and that abnormal expression of this protein may contribute to the progression of breast and ovarian cancer. In support of this hypothesis, we have observed that exogenous expression of g-synuclein in tumor cells elicits a phenotype similar to that induced by activation of the Rho/Rac/CDC42 pathway, i.e., altering the appearance of focal adhesions and stress fibers, and enhancing motility and invasion. Strikingly, levels of activated Rac (GTP-bound) are constitutively elevated in ovarian tumor cells that overexpress g-synuclein. Synuclein proteins also exhibit a weak homology to the 14-3-3 family of cytoplasmic chaperone proteins. 14-3-3 proteins help regulate different signal transduction pathways by directly binding to various protein kinases and bringing them into close proximity with substrate and regulatory proteins. We have demonstrated a novel interaction of g-synuclein with the MAPKs, ERK1/2 and JNK1, in a single complex. Overexpression of g-synuclein leads to increased ERK activation but does not activate JNK. As activated ERK1/2 specifically localize to focal adhesions, and ERK1/2 has been shown to enhance migration, these results raise the possibility that g-synuclein may enhance the metastatic potential of tumors through the activation of Rac within the Rho signaling pathway based on protein interactions at focal adhesions. We hypothesize that g-synuclein contributes to tumorigenesis by promoting cell motility as a result of altering the Rho and ERK1/2 signaling pathways and that g-synuclein may be an important therapeutic target.

**Gene expression profile comparison between tumorigenic and non-tumorigenic clones from ovarian cancer cells.**

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Ovarian cancer is the leading cause of death from gynecological neoplasias. The molecular events involving the initiation and progression of ovarian cancer are not well defined. In this report we describe the utilization of a set of eight Human GeneFilters Microarrays containing > 30000 human genes to define the differences in gene expression between tumorigenic and non-tumorigenic hybrid clones derived from a human ovarian carcinoma cell line SKOV-3 with the introduction of human chromosome 11. A total of 26 differentially expressed genes with expression levels varied significantly by 2- or more fold were identified, of which three genes, lipocalin 2 (oncogene 24p3)(LCN2), DNA-damage -inducible transcript 3 (DDIT3) and prominin (mouse)-like 1 (PROML1), were up-regulated in tumorigenic hybrid clones. Among the 23 down-regulated genes in tumorigenic hybrid clones, spleen tyrosine kinase (SYK) and caveolin 1 (CAV1) have been proposed as tumor suppressor genes, while Thy-1 cell surface antigen (THY1) has been identified as a putative tumor suppressor gene for ovarian cancer in a previous study in our laboratory.



**Transfer of the FHIT gene induces apoptosis in the lung cancer cell line H460 through a caspase-8-dependent and mitochondria-independent mechanism.** *L. Roz<sup>1</sup>, C.G. Ferreira<sup>2</sup>, H. Ishii<sup>3</sup>, C.M. Croce<sup>3</sup>, G. Giaccone<sup>2</sup>, G. Sozzi<sup>1</sup>.*

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Chromosome 3p alterations are one of the most frequent genetic changes associated with the development of lung cancers and the FHIT gene, located at 3p14.3, is often inactivated in these tumors. We have shown previously that adenoviral-mediated restoration of Fhit expression in non-small cell lung cancer (NSCLC) cell lines results in apoptosis and loss of tumorigenicity in nude mice through a mechanism involving caspase-8 activation. In order to investigate how this effect is achieved we used different clones of the NSCLC cell line H460 with alterations in genes involved in the regulation of the apoptotic pathway. These clones were transduced with an adenoviral vector expressing the FHIT gene and the apoptotic response was monitored using the TUNEL assay. The same clones were also treated with cytotoxic drugs to analyse their response to other apoptotic stimuli. A complete protection from Fhit-induced apoptosis was seen in clones transfected with a dominant negative form of FADD and with the viral inhibitor of caspase-1 and caspase-8 CrmA (while an inactive mutant form of CrmA didn't confer any resistance). On the contrary clones overexpressing Bcl-2 or Bcl-x<sub>L</sub>, that are protected from cisplatin, topotecan and gemcitabine induced apoptosis, remained highly susceptible to FHIT transfer. Taken together these data seem to indicate that in H460 cells the mechanism of action of Fhit mainly involves activation of a FADD-related cytoplasmic apoptotic pathway, without mitochondrial amplification of the response. Interestingly this is different from the results observed with chemotherapy-induced apoptosis (which requires mitochondrial intervention) suggesting a possible role of Fhit in facilitating caspase-8 activation at the cytoplasmic level.

**Prune and nm23-H1 and nm-23 H2 (NDP-Kinase) proteins: involvement in cancer.** *M. Zollo<sup>1</sup>, A. D'Angelo<sup>1</sup>, A. Andre'<sup>1</sup>, V. Aglio<sup>1</sup>, S. Olivieri<sup>2</sup>, G. Arrigoni<sup>2</sup>, A. Forus<sup>3</sup>, O. Myklebost<sup>3</sup>, A. Ballabio<sup>1</sup>.* 1) TIGEM, Telethon Institute of Genetics and Medicine, Naples, Italy; 2) Department of Pathology, San Raffaele Hospital (HSR), Milan, Italy; 3) Department of Tumour Biology, The Norwegian Radium Hospital, Oslo, Norway.

We have isolated the human and murine homolog of the *Drosophila* prune gene through dbEST searches 1,3. In mouse, Prune is expressed (in situ analysis) at low levels in the basal plate along the entire neural tube while at E12.5 the expression in the nervous system is definitively stronger, especially in the cranial and dorsal root ganglia and in the spinal nerves. In adult mouse and human tissues its expression is ubiquitously. Furthermore, in human adult brain tissues (IHC analysis) prune protein is present mainly on neurons and astrocytes cells. By using yeast interaction-mating and "in vitro" and "in vivo" co-immuno-precipitation experiments, we show the ability of human prune to interact with the human nm23-H1,2,3 and nm23-H2 (metastasis suppressor genes), and demonstrated that is impaired with nm-23-H1-S120G mutant, a gain-of-function mutation associated with advanced neuroblastoma. Prune protein retains the four characteristic domains of DHH phosphoesterases<sup>4</sup> and we are showing that is a new phosphodiesterase (PDE) specific for cAMP substrate. In addition, in Sarcoma tumours (MFH, MS, LS, LMS) we have found PRUNE is amplified (3-8 copies) by FISH and CGH analysis and these findings correlates at protein levels (IHC analysis). Infection of NIH3T3 cells with a PRUNE recombinant retrovirus increased cell proliferation. Possibly, amplification and overexpression of PRUNE has the same effect in the tumours. Our working hypothesis is that prune is negative regulating the anti-metastatic function of nm23 protein. REFERENCES 1. Banfi, S., et al. *Nature Genetics* 13, 167-174 (1996). 2. Hartsough, M.T. & Steeg, P.S. *Am J Hum Genet* 63, 6-10 (1998). 17-19 (1998). 3. Reymond, A., et al. *Oncogene* 18, 7244-52 (1999). 4. Aravind, L. & Koonin, E.V. *Trends in Biochemical Sciences* 23,.

**Expression profiles of uveal melanomas with good and poor prognosis.** *F. Tschentscher, B. Horsthemke, D.R. Lohmann, M. Zeschnigk.* Institut fuer Humangenetik, Universitaetsklinikum, Essen, Germany.

Uveal melanoma is the most common primary intraocular tumor with an incidence of 6 per 1 million Caucasians per year. Cytogenetic analysis and comparative genomic hybridization have revealed nonrandom aberrations of chromosomes 3, 6 and 8. Monosomy 3 (ms3), which is frequently accompanied by gain of 8q material and found in about 50 % of the tumors, significantly predicts metastatic disease and poor prognosis. Conversely, tumors with disomy 3 (ds3) rarely give rise to metastatic disease. To obtain information about the molecular mechanisms underlying this striking difference in clinical behavior, we compared the expression profiles of 9 and 10 primary uveal melanomas with ms3 and ds3, respectively, using oligonucleotide arrays with probes for more than 1700 cancer related genes (Affymetrix Human Cancer G110). After exclusion of genes showing no variation in expression, a two-way hierarchical clustering algorithm was applied, by which the tumors were grouped in two distinct clusters. One contains 8 tumors with ms3 and 2 tumors with ds3, while the other consists of 8 tumors with ds3 and one tumor with ms3. Visual inspection of the expression map showed two clusters of genes with pronounced differences in expression. Cluster 1 includes 75 genes showing decreased expression in ms3 tumors compared to ds3 tumors. Most of them are not located on chromosome 3. Cluster 2 includes 24 genes showing decreased expression in ds3 tumors compared to ms3 tumors. Therefore, the expression profile of these genes correlates well with the two genetically defined classes of tumors with distinct metastatic potential. Further analyses of the differentially expressed genes may help to unravel the complex process of metastatic behavior.

**Tetracycline-regulated expression of the candidate tumor suppressor gene, RASSF1A, from a frequently affected chromosomal region 3p21.3.** A. Protopopov<sup>1</sup>, J. Li<sup>1</sup>, V. Kashuba<sup>1</sup>, K. Dreijerink<sup>2</sup>, E. Braga<sup>3</sup>, I. Kuzmin<sup>2</sup>, M. Lerman<sup>2</sup>, E. Zabarovsky<sup>1</sup>. 1) Microbiol & Tumor Biol Ctr, Karolinska Inst, Stockholm, Sweden; 2) Lab Immunobiol & Intramural Res Support Program, SAIC, NCI Frederick, MD; 3) Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia.

Clear cell type renal cell carcinomas (clear RCC) are almost universally characterized by loss of heterozygosity (LOH) on chromosome 3p, which usually involves any combination of three regions: 3p25-p26 (harboring the VHL gene), 3p12-p14.2 (containing the FHIT gene) and 3p21-p22, implying inactivation of the resident tumor suppressor genes. For the 3p21-p22 region, the affected TSGs remain, at present, unknown. Recently, the RAS association family 1 gene (isoform RASSF1A), located at 3p21.3, has been identified as a candidate lung and breast TSG. We found hypermethylation of RASSF1A's GC-rich putative promoter region in most of analyzed samples, including 39 of 43 primary tumors (91%). The promoter was partially or completely methylated in all 18 clear RCC cell lines analyzed. Methylation of the GC-rich putative RASSF1A promoter Forced expression of RASSF1A transcripts in KRC/Y, a renal carcinoma cell line containing a normal and expressed VHL gene, suppressed growth on plastic dishes and anchorage-independent colony formation in soft agar. Mutant RASSF1A had significantly reduced growth suppression activity. These data suggest that RASSF1A is the candidate renal TSG gene for the 3p21.3 region. In subsequent experiments the concentration of tTA-effector (doxycycline) will be varied so as to enable estimation of the level of the gene expression required for effects on tumor cells. Engineering of SCLC and other cell lines with controlled levels of RASSF1A expression is also in the pipeline.

**Ovarian germ-cell tumor occurring in a *BRCA2* mutation carrier.** *N. Wong*<sup>1,2</sup>, *N. Hamel*<sup>6</sup>, *A. Péroquin*<sup>4</sup>, *M. Al-Saffar*<sup>5</sup>, *W.D. Foulkes*<sup>1,2,3,5,6</sup>. 1) Cancer Prevention Research Unit, SMBD-Jewish General Hospital; 2) Dept. of Oncology, SMBD-Jewish General Hospital; 3) Dept of Medicine, SMBD-Jewish General Hospital; 4) Dept. of Pathology, SMBD-Jewish General Hospital; 5) Dept. of Human Genetics, McGill University Health Centre; 6) Research Institute of the McGill University Health Centre.

Germ-cell tumors (GCT) account for about 20% of neoplastic disease of the ovary with only about 2 to 3% of GCT being malignant. *BRCA*-linked ovarian cancers are usually epithelial tumors, the more common type of ovarian cancer. We report the occurrence of a malignant ovarian GCT in an Ashkenazi Jewish woman who was recently found to carry a 6174delT *BRCA2* mutation. At age 33, six months following a normal vaginal delivery, the patient presented in 1985 with a pelvic mass and an elevated serum bHCG. She underwent a TAH-BSO, omentectomy with paraortic lymph node dissection. Pathological examination revealed a stage Ia mixed GCT in the left ovary: 50% embryonal carcinoma, 20-25% choriocarcinoma, 10-15% dysgerminoma and 10-15% immature teratoma. The tumor measured 9 x 7 x 6 cm with a multinodular surface appearance. There was no evidence of metastatic disease and peritoneal washings were negative. She was treated with 3 cycles of a combination of cyclophosphamide, actinomycin D, bleomycin, vinblastin and cisplatin. She did not receive radiation therapy. Patient has remained disease free. To assess the involvement of *BRCA2* in the GCT, LOH analysis was performed on the tumor using a marker (D13S1698) thought to be 70kb proximal to *BRCA2*. There was no evidence of LOH at this marker in the sample of dissected tumor. We believe that this is the first report of a GCT in a *BRCA2* mutation carrier. Given the infrequency of GCTs, even if the relative risk for developing it is the same as for ovarian carcinoma, GCTs are still likely to occur rarely in mutation carriers. There has been one report of a *BRCA1* mutation carrier who developed a non-epithelial malignant ovarian tumor. Further studies are needed to determine if *BRCA* genes are involved in the development of GCT or if its occurrence in mutation carriers is an unrelated chance event.

**Expression profiling of medulloblastoma: identification of therapeutic targets for metastatic disease. K.M.**

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Metastatic medulloblastoma is highly associated with poor outcome and little is known about the genetic regulation of medulloblastoma dissemination. Whole-brain and spine radiation therapies, which are used to prevent metastases, are non-specific and result in lowered IQ, hearing loss and growth retardation. We used expression profiling to identify molecular targets and match agents to better prevent metastatic disease. We expression profiled 23 primary medulloblastomas clinically designated as either metastatic (M+) or non-metastatic (M0) and identified 85 genes whose expression differed significantly between classes. A class prediction algorithm based on these genes assigned sample class to these tumors (M+ or M0) with 72% accuracy using a leave-one-out approach and to four additional independent tumors with a 100% accuracy. Class prediction also assigned the metastatic medulloblastoma cell line Daoy to the metastatic class. Notably upregulated in the M+ tumors were platelet derived growth factor receptor alpha (*PDGFRA*) and members of the downstream RAS/MAPK signal transduction pathway. Immunohistochemical validation on an independent set of tumors showed significant overexpression of *PDGFRA* in M+ tumors as compared to M0 tumors. Using *in vitro* assays, we showed that PDGFA enhances medulloblastoma migration and increases downstream MEK1/2 and MAPK phosphorylation in a dose-dependent manner. Finally, neutralizing antibodies to *PDGFRA*, or U0126, a highly specific chemical inhibitor of MEK1/2, blocked MEK1/2 and MAPK phosphorylation, inhibited migration, and prevented PDGFA-stimulated migration. These results provide the first insight into the genetic regulation of medulloblastoma metastasis and are the first to suggest a role for *PDGFRA* and the RAS/MAPK signaling pathway in medulloblastoma metastasis. Inhibitors of *PDGFRA* and RAS are being considered as possible novel therapeutic strategies against medulloblastoma.

**Characterization of drug resistant malignant melanoma cells by gene expression profiling.** *R. Wittig<sup>1</sup>, M. Nessler<sup>2</sup>, B. Korn<sup>3</sup>, P. Lichter<sup>2</sup>, D. Schadendorf<sup>4</sup>, A. Poustka<sup>1</sup>.* 1) Molecular Genome Analysis, German Cancer Research Center (DKFZ), Heidelberg, Germany; 2) Organisation of Complex Genomes, DKFZ, Heidelberg, Germany; 3) Ressource Center for Genome Research (RZPD), Heidelberg, Germany; 4) Clinical Cooperation Unit "Dermatooncology", DKFZ, Heidelberg, Germany.

Drug resistance of tumor cells often leads to severe problems in the treatment of cancer. We examined differences in mRNA expression patterns between a drug sensitive and 3 derived drug resistant malignant melanoma cell lines with acquired resistance to the DNA damaging agents cisplatin, etoposide and fotemustine, respectively. In order to find genes which contribute to the drug resistant phenotype the expression of about 31.500 gene and EST sequences represented in a UniGene clone collection (Human UniGene Set RZPD1) was analysed by comparative cDNA array hybridisation. Cells resistant to cisplatin and etoposide revealed strong upregulation of interferon inducible genes. Some differentially expressed genes were formerly found to be involved in stress response and cell death processes, others are less characterised or even unknown. Single genes are up- or downregulated in more than 1 resistant cell line, indicating a contribution to observed cross resistance against different drugs. Etoposide resistant cells showed the most remarkable differences. This correlates very well with the high relative degree of etoposide resistance and with the elevated number of genetic alterations, which could be confirmed by comparative genomic hybridisation. We verified the differential expression of several chemoresistance candidate genes by Northernblot analysis. Now we developed a cDNA array harboring 170 candidate genes in order to find out their importance for drug resistance in general by gene expression profiling studies in other experimental systems.

**Polymorphic Variants of Nuclear Receptor Genes Associated with Sporadic Breast Cancer.** *J.E. Curran<sup>1</sup>, S.R. Weinstein<sup>2</sup>, L.R. Griffiths<sup>1</sup>*. 1) Genomics Research Centre, Griffith University Gold Coast, Southport; 2) Pathology Department, Gold Coast Hospital, Southport, Queensland, Australia.

A cross-sectional association approach has been used in many previous studies to detect genes involved in disease development. We are using such an approach to investigate sporadic breast cancer. Recent results from our laboratory have implicated polymorphisms within the 3' region of the vitamin D receptor gene (VDR)1 as being involved in this common form of cancer. These results have prompted further investigation of other nuclear receptor genes. There is increasing evidence that genetic variations in nuclear receptor genes can cause loss of hormone dependency in different cancer types and may lead to inappropriate gene functions or tumour development. In this study, polymorphisms within the glucocorticoid (GRL), estrogen (ESR), progesterone (PgR) and androgen receptors (AR) were examined in an age and ethnic matched breast cancer affected and control population.

When tested in the case-control populations, results indicated association of tested polymorphisms of glucocorticoid and estrogen receptors with sporadic breast cancer<sup>2</sup>. A highly polymorphic dinucleotide repeat, D5S207, located within 200kb of GRL showed a significant variation in allele frequencies among populations ( $P=0.001$ ), while a codon 594 (exon 8) ESR polymorphism also showed a significant association with sporadic breast cancer ( $P = 0.005$ ). We are presently investigating other polymorphisms within these genes as well as the progesterone and androgen receptor genes for association. Our results suggest a role for nuclear receptor genes in sporadic breast cancer and justify further investigation of the role of these and other nuclear receptors in the aetiology of this disorder.

References:

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**CAG repeat length in the gene AIB1 gene modifies breast cancer risk in BRCA1/2 mutation carriers.** Z. Kote-Jarai<sup>1</sup>, L. Kadouri<sup>2</sup>, F. Durocher<sup>3</sup>, D.F. Easton<sup>3</sup>, A. Huber<sup>2</sup>, D. Abeliovich<sup>2</sup>, B. Glaser<sup>2</sup>, T. Peretz<sup>2</sup>, R. Eeles<sup>1</sup>. 1) Cancer Genetics, The Institute of Cancer Research, Sutton, UK; 2) Hadassah Medical Center, Jerusalem Israel; 3) CRC Genetic Epidemiology Unit, Cambridge, UK.

Variation in the penetrance estimates for BRCA1 and BRCA2 mutation carriers suggests that genetic polymorphisms may modify the cancer risk in carriers. Polymorphisms of genes involved in hormonal signal transduction are possible candidates. The AIB1 gene, an estrogen receptor (ER) coactivator, is frequently amplified in breast and ovarian tumors. Variation of a CAG repeat length, that encodes a polyglutamine repeat in the C-terminus of the protein, has been reported. 188 BRCA1/2 mutation carriers, (of which 156 were of Ashkenazi origin) and 156 unaffected control individuals of Ashkenazi origin, aged over 56, were genotyped for the AIB1 CAG repeat. Risk analyses were conducted using a variant of the log rank test assuming penetrance levels appropriate to the Ashkenazi population. For BRCA1/2 carriers the estimated breast cancer (BC) risk (RR) per average repeat length adjusted for population (Ashkenazi, non-Ashkenazi) was 1.56 (95%CI 1.09-2.24, p=.014). When analysis was restricted to BRCA1 carriers RR was 1.92 (95%CI 1.23-3.02, p=.004). For individuals with 2 alleles <sup>3</sup>15 CAG repeats RR of BC was 1.93 (95%CI 0.98-3.79) and 0.55 (95%CI 0.28-1.08) for those with at least one allele £12 repeats. The average age of onset was also significantly lower for individuals carrying two alleles <sup>3</sup>15 repeats (39.9 years) compared to individuals with at least one short allele of £12 repeats (48.1 years), p=.001. These results indicate elevated risk for BC in carriers of BRCA1/2 mutations with a longer CAG repeat of the AIB1 gene, especially in carriers of both alleles with <sup>3</sup>15 CAG repeats, which comprise 20% of our carrier population. In conclusion, our results suggest that the AIB1 CAG repeat length could be an important predictor of breast cancer risk among women with mutations in the highly penetrant BRCA1/2 genes.

**3'UTR polymorphisms in the CDKN2A gene and age at onset of familial and sporadic melanoma from Liguria.**

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The common polymorphisms 540C®G and 580C®T in the 3'UTR of the CDKN2A (p16) gene, whose mutations are known to confer susceptibility to cutaneous malignant melanoma, have been previously studied but their influence on melanoma risk is currently debated. In particular an Australian population-based study found an association between familial risk and the 540G allele, possibly influenced by Celtic ancestry, and no association with 580T. In this study we performed molecular analysis of 255 melanoma patients from 751 familial and sporadic Ligurian patients referred to the outpatients clinic of the National Cancer Institute (IST) and the Medical Genetics Service of Genoa over a 6-year period (1/1/1995-31/12/2000). Because our goal was to establish the relevance of CDKN2A polymorphisms in Ligurian melanoma patients according to age at diagnosis we split the 751 cases into quartiles based on age at diagnosis (17-40, 41-53, 54-64, and 65-88 yr) and compared the prevalence of polymorphisms in each sub-set. A small group (67) of healthy Ligurian spouses of familial and sporadic patients served as geographical controls. The 540G allele does not seem to influence age at onset: I vs. IV, I vs. II,III,IV (Chi squares n.s). We found a higher frequency of the 580T allele in quartile IV vs. quartile I (p=0.024), vs. quartiles I,II,III (p=0.04) and vs. controls (p=0.045). When we looked at sporadic and familial melanoma separately we found the same trend with no statistical significance (p=0.077, 0.072 respectively). The 580T allele seems to be associated with a late age of onset of the disease in our Ligurian population, which is characterized by a recurrent mutation (G101W) due to a founder effect, but we can't exclude an eventual protective modifier action. This study was supported by Ministry of Health grants N.ICS. 070.1/RF/00.184 to GBS.

**APC E1317Q variant is not associated with increased colorectal neoplasia risk.** *G.M. Petersen, D. Hahnloser, K. Rabe, K. Snow, L. Boardman, N.M. Lindor, B. Koch, L. Wang, S.N. Thibodeau.* Mayo Clinic, Rochester, MN.

Genetic susceptibility may play a role in many colorectal cancers (CRC). Known syndromes such as familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) account for <5% of CRC. The APC variant, E1317Q, caused by G to C mutation, has been reported to be associated with increased risk of colorectal polyps and CRC; these findings are controversial. **Aim:** To evaluate whether APC E1317Q is associated with increased risk of adenomatous polyps and CRC. **Methods:** Data on clinical and family history of cancer and blood samples were prospectively collected from 6 groups of Caucasian subjects at Mayo Clinic: A) Unselected CRC, n=319, mean age 66; B) Colorectal adenomas (1 to 3), n=52, mean age 65; C) Attenuated polyposis, n=141, mean age 42; D) CRC  $\leq$  age 50 (FAP/HNPCC excluded), n=126, mean age 43; E) Asymptomatic persons with normal colonoscopy, n=201, mean age 60; and F) spouse controls of CRC patients, n=451, mean age 60. E1317Q genotyping was done on lymphocyte DNA by Pyrosequencing©, blind to disease status. **Results:** The frequency of heterozygotes for E1317Q among colorectal neoplasia patients did not differ from controls; compared to Group F (0.022), there were no statistically significant differences for Groups A (0.028,  $c^2$  test,  $p=0.6$ ), B (0.058, Fisher's exact,  $p=0.14$ ), or C (0.007, Fisher's exact,  $p=0.47$ ), though the latter two were smaller samples. No one in Group E carried E1317Q. When examined by age at onset, there was no difference in frequency of E1317Q among younger CRC (Group A age  $\leq 50$  plus Group D (0.013); n=157) vs. older CRC (Group A  $>$ age 50 (0.028); n=288),  $p=0.51$ , nor when compared to Group F ( $\leq$ age 50 (0.0), n=98,  $p=.53$ ;  $>$  age 50 (0.028), n=352,  $p=.96$ ). Similarly, no differences were found by family history of CRC in Group A (family history positive (0.036), n=56, vs no family history (0.027), n=263),  $p=0.66$ . **Conclusions:** The rare variant, APC E1317Q, does not appear to be associated with increased colorectal neoplasia risk. *Supported in part by NIH U01 CA74800 (Cooperative Family Registry for Colon Cancer Studies)..*

**Reproductive factors and ovarian cancer risk in Jewish *BRCA1* and *BRCA2* mutation carriers.** *R. Moslehi*<sup>1</sup>, *F. Modugno*<sup>2</sup>, *R. Ness*<sup>2</sup>, *S. Narod*<sup>3</sup>. 1) Division of Cancer Epidemiology & Genetics, NCI, NIH, Bethesda, MD; 2) Department of Epidemiology, University of Pittsburgh, PA; 3) Centre for Research on Women's Health, Women's College Hospital, University of Toronto, ON.

Oral contraceptive use, childbearing, breastfeeding and tubal ligation have been shown to protect women in the general population against epithelial ovarian cancer. To determine whether these factors are also protective in women carrying mutations in the cancer predisposing genes, *BRCA1* and *BRCA2*, we performed a case-only study on 242 Jewish women with invasive epithelial ovarian cancer. All were genotyped for Ashkenazi founder mutations (185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*). We compared oral contraceptive (OC) use, childbearing, breastfeeding, gynecologic surgeries and other reproductive factors in carriers and non-carriers, using unconditional logistic-regression. Among the 242 cases, 64 carried one of the *BRCA1* founder mutations, and 31 carried the *BRCA2* mutation. Parous *BRCA1* carriers reported fewer live births than non-carriers (average of 2.1 versus 2.5 live births,  $P < 0.02$ ). Parous *BRCA2* carriers also reported fewer live births (average of 2.3 live births) although the difference was not significant. Carriers and non-carriers did not differ in their history of breastfeeding or lifetime use of OC. However, *BRCA1* carriers began OCs at a later mean age (24.0 years versus 23.2 years of age,  $P < 0.04$ ) and were also more likely to be recent users of OCs (average 19.6 versus 21.4 years since last use,  $P = 0.04$ ). *BRCA1* carriers were more likely than non-carriers to have had a tubal ligation (25.0% versus 10.2%,  $P < 0.01$ ). Oral contraceptive use, childbearing and breastfeeding appear to protect *BRCA1* and *BRCA2* carriers from ovarian cancer. The late age of OC initiation reported by cases carrying a *BRCA1* mutation suggests that the protection from OCs maybe more effective at an early age. The higher percentage of *BRCA1* carriers reporting a tubal ligation suggests that this procedure may not be as protective for carriers as it is for non-carriers.

**Prevalence of inherited mutations in BRCA2 in a population-based series of 265 African American breast cancer patients.** *R.K. Kim<sup>1</sup>, T. Walsh<sup>1</sup>, H. Mu<sup>1</sup>, B. Newman<sup>2</sup>, M-C. King<sup>1</sup>.* 1) Depts of Medicine and of Genomic Sciences, University of Washington, Seattle, WA; 2) School of Public Health, Queensland University of Technology, Australia,.

To define the prevalence of disease-predisposing BRCA2 mutations among African American women with breast cancer, we analyzed genomic DNA from 265 patients from the population-based Carolina Breast Cancer Study (CBCS). The entire coding region and flanking intronic and 5' and 3' UTR sequences of BRCA2 were screened by protein truncation test and single-stranded conformation polymorphism, followed by DNA sequencing of variant bands. BRCA2 mutations that definitely lead to protein truncation were identified in three patients (1.13%). The mutations were 7180 C to T, a nonsense mutation at codon 2318 in exon 13; 7772 insA, leading to a stop at codon 2523; and 10173 delA, leading to a stop at codon 3317. A fourth mutation in the intron 14 splice donor may also be deleterious but its effect could not be evaluated from genomic DNA. Other amino acid substitutions, silent SNPS, and mutations in the 5' and 3' UTRs were also identified. None of the BRCA2 mutations previously reported in African American families occurred in the series. Two features of the distribution of BRCA2 mutations in this series are intriguing. First, none of the mutations we found had been reported previously among African American families (one was reported independently in a family from Sweden). Second the proportion of breast cancer attributable to BRCA2 among African American women was lower than among Caucasian breast cancer patients from the same CBCS series who were screened in the same way.

**Unusual alternative splicing within the human kallikrein genes KLK2 and KLK3 gives rise to novel prostate-specific proteins.** *L. Mintz*<sup>1</sup>, *J. Bernstein*<sup>2</sup>, *Z. Levine*<sup>2</sup>, *K. Savitsky*<sup>2</sup>, *A. David*<sup>2</sup>, *I. Azar*<sup>2</sup>, *S. Engel*<sup>2</sup>, *R. Hosrabi*<sup>2</sup>, *A. Toporik*<sup>2</sup>, *J. Romano*<sup>2</sup>. 1) Compugen Inc., Jamesburg, NJ; 2) Compugen Ltd., Tel-Aviv, Israel.

Prostate-specific antigen (PSA) is considered the best cancer biomarker currently available, and is widely used for screening, diagnosis and monitoring of prostate cancer. PSA (also named human kallikrein 3, hK3) is encoded by the KLK3 gene, a member of the human kallikrein gene family. The kallikrein genes code for a group of serine proteases involved in post-translational processing events. We report here the identification of two novel polypeptides encoded by unusual mRNA splice variants of the KLK2 and KLK3 genes. These proteins, named PSA-linked molecule (PSA-LM) and hK2-linked molecule (K-LM), share only the signal peptide with the original protein product of the respective gene. The mature proteins, however, are entirely different and have no similarity to the kallikrein family or to other proteins in the databases. Similar to PSA and hK2, PSA-LM and K-LM are expressed in the secretory epithelial cells of the prostate and are upregulated in response to androgenic stimulation.

**The human endogenous retrovirus HERV-K in prostate cancer.** *R.D. Smith<sup>1,2</sup>, B. Khoubehi<sup>1,2</sup>, J.D. Beatty<sup>2</sup>, C.W. Ogden<sup>2</sup>, M.A. Penny<sup>1</sup>.* 1) Department of Medical and Community Genetics, Imperial College of Science, Technology and Medicine, London, UK; 2) Department of Urology, Northwick Park Hospital, Harrow, UK.

The ability of human endogenous retroviruses (HERVs) to retrotranspose has led to the hypothesis that their expression may have a role in carcinogenesis: 1 person in 8 may carry an endogenous insertional mutation due to a new retrotransposition event. Although their transcription is usually silenced by methylation, HERV expression has been identified in a number of malignancies including breast and colon cancer. HERV-K transcription is up-regulated by steroid hormones in the breast cancer cell line T47D. Prostate cancer is also sensitive to its hormonal environment, raising the possibility of similar regulation of HERV-K expression. We have therefore investigated HERV-K expression and possible retrotransposition in prostate cancer.

RNA and DNA were extracted from DU-145, PC3 and LNCaP prostate cancer cell lines, 27 prostate cancer (CaP) and 26 benign prostatic hyperplasia (BPH) specimens. HERV-K expression was analysed using an RT-PCR technique developed to amplify mRNA without amplifying genomic HERV sequences. cDNA sequencing and Southern hybridisation confirmed the results. HERV-K integration sites in the genomic DNA of these samples were compared by restriction enzyme analysis and Southern hybridisation with the fragment pattern of 40 control men.

HERV-K *envelope* expression was detected in the T47D positive control, all prostate cell lines, 16/27 CaP specimens (59%) and 7/26 BPH specimens (27%) ( $p=0.02$ , 2-tailed Fishers exact test). Different HERV-K restriction fragments were identified in the cell lines and CaP specimens that were not present in controls.

Our results suggest that HERV-K expression occurs in prostate tissue, and is significantly more prevalent in CaP than in BPH. Differences in the restriction fragment patterns could be the result of expressed HERVs acting as mobile elements with the potential to disrupt the function of tumour suppressor genes or enhance expression of proto-oncogenes via promoter activity in the Long Terminal Repeats (LTRs) of the viral sequence.

**Different microsatellite mutation rates in cancer cell lines with defects in different mismatch repair genes.** N.A. Yamada, A. Castro, R.A. Farber. Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The mismatch repair (MMR) system is an evolutionarily conserved DNA repair pathway, which is critical for the maintenance of a stable genome. Replication errors, including insertion-deletion loops in microsatellites, are corrected by this repair system, and microsatellite mutation rates are significantly elevated in the absence of MMR. Hereditary nonpolyposis colorectal cancer (HNPCC) is a cancer predisposition syndrome that results from defective MMR, and a significant fraction of related sporadic tumors have somatic MMR defects. There are two known major protein complexes in the human MMR pathway, hMutS and hMutL. The human MutLa complex is composed of hMLH1 and hPMS2. HCT116 is a colorectal cancer cell line with defects in the *hMLH1* gene, and HEC-1-A is an endometrial cancer cell line with defects in the *hPMS2* and *hMSH6* genes. A plasmid vector containing a (CA)<sub>17</sub> microsatellite was stably transfected into each cell line, where it integrated into the genome. The microsatellite sequence was placed upstream of the bacterial neomycin resistance gene (*neo*), such that the *neo* reading frame was disrupted. Clones with frameshift mutations that restored the normal reading frame of the *neo* gene were selected, and mutation rates were determined by fluctuation analysis. Microsatellite mutation rates were approximately ten-fold higher in HCT116 than in HEC-1-A; the majority of mutations in both cell lines were deletions of a single repeat unit. These findings indicate that the absence of hMLH1 may have a greater effect on microsatellite mutation rates than the absence of hPMS2 (even in combination with the absence of normal hMSH6). The similarity in types of mutations suggests that the difference in mutation rates is not the result of a qualitative difference in the roles that the two proteins play in MMR, but of the greater reduction in overall efficiency of MMR in HCT116. Alternatively, HCT116 may harbor mutations in additional genes that affect repair or the fidelity of replication, which could lead to the higher mutation rate of this cell line than that of HEC-1-A.



**Valproic Acid Embryopathy: Report of Two Siblings with Further Expansion of the Phenotypic Abnormalities and a Review of the Literature.** *C. Kozma*. Dept Pediatrics, Georgetown Univ, Washington, DC.

Fetal Valproate syndrome (FVS) results from prenatal exposure to Valproic acid (VPA). It is characterized by a distinctive facial appearance, a cluster of minor and major anomalies, and central nervous system dysfunction. In this report, two siblings who were exposed to monotherapy with VPA are described with documentation of long-term follow up. Both children had craniofacial findings, multiple systemic and orthopedic abnormalities, an overgrowth pattern, and developmental deficits. The literature from 1978 to 2000 is reviewed. A total of 69 cases that were solely exposed to VPA with adequate phenotypic description were identified. The clinical manifestations of FVS encompass a wide spectrum of abnormalities including consistent facial phenotype, multiple systemic and orthopedic involvement, central nervous system dysfunction, and altered physical growth. The facial appearance is characterized by a small broad nose, small ears, flat philtrum, a long upper lip with shallow philtrum, and micro/retrognathia. In this review, 62% of the patients had musculoskeletal abnormalities, 30% had minor skin defects, 26% had cardiovascular abnormalities, 22% had genital abnormalities, and 16% had pulmonary abnormalities. Less frequently encountered abnormalities included brain, eye, kidney, and hearing defects. Neural tube defects were seen in 3% of the sample. Twelve percent of affected children died in infancy and 29% of surviving patients had developmental deficits/mental retardation. While 15% of patients had growth retardation, an overgrowth pattern was seen in 9%. The data from this comprehensive review especially the developmental outcome should be added to the teratogenic risk, which arises in association with the use of VPA during pregnancy.

**A possible Y-linked nonsyndromic hereditary progressive hearing loss found in a large six generations Chinese pedigree.** *Q. Wang<sup>1</sup>, W. Yang<sup>1</sup>, N. Li<sup>2</sup>, J. Cao<sup>1</sup>, L. Yu<sup>1</sup>, D. Han<sup>1</sup>, S. Jiang<sup>1</sup>.* 1) Otolaryngology/Head/Neck Surg, PLA Otolaryngology Inst, Beijing, China; 2) National Laboratories for Agrobiotechnology, Beijing, China.

A Y-linked inheritance pattern of nonsyndromic hereditary hearing loss was found in a six-generation family from the Southern of China. The phenotype of hearing loss was only observed in patrilineal males in every generation. Females and their offspring were not affected. We assume the Y-linked inheritance pattern existed in the pedigree. The ancestor of this family can trace back to eight hundreds years ago, who settled down the town of Jiang Xi province, the Southern of China. The earliest record of hearing-impaired family member was born in 120 years ago. He died just before his first child was born. The child (son) was affected hearing loss at about age 20. He got married and had four boys and one girl. The children then were subdivided into five branches in our study. They lived in three different areas after they got marry. The first branch had ten patrilineal males in following three generations and all affected. The second branch had one boy and affected. He died before age 40. The third branch had seven patrilineal males and all affected. The fourth branch had six patrilineal affected males. Their disease phenotypes were postlingual with moderately to severe hearing loss. The last branch was from the only girl in the third generation. She had two boys. Their hearing was normal at age 46 and 58 tested on April of year 2000. Their following generations were normal. We investigated 113 individuals and found 30 affected males in the family. 94 members are alive. 38 members accepted audiometry (including PTA, ABR, Immittance testing). Blood samples were obtained from the 38 family member after informed consent, which are from 16 patrilineal affected males, 4 maternal normal male and 18 normal females. The hearing loss in family affected members was extremely similar to each other, which showed a slope down pattern from 500Hz to 8000 Hz, more severe in high frequency. From the clinical characterizations, we are coming to conclude that Y-linked inheritance pattern presented in the pedigree.

**Another patient with COFS syndrome due to a nucleotide excision repair (NER) defect.** *A. Brooks, G. Mancini, W. Kleijer, P. Willems.* Dept of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

We report on two girls with a low birth weight and a small head circumference born to healthy, consanguineous parents of Moroccan origin. Growth remained below the 3rd centile. The youngest daughter also showed a high nasal bridge, bilateral microphthalmia, bilateral cataract and contractures of the hands. On sun exposure at the age of 9 months she developed a severe hypersensitive skin reaction with erythema. The eldest daughter was noted to have flexion contractures of the large joints. She died at the age of 8 months due to severe failure to thrive and aspiration pneumonia. Autopsy was not performed. The combination of microcephaly, cataracts and joint contractures suggested the clinical diagnosis of Cerebro-Oculo-Facial-Skeletal syndrome (COFS). Given the established clinical overlap with Cockayne syndrome, which is caused by mutations in genes involved in nucleotide excision repair (NER), DNA repair studies of UV- exposed cultured fibroblasts were performed in the youngest patient. DNA replication after exposure to a UV dose of  $6 \text{ J/m}^2$  was abnormally depressed (10% of the control). This case report underlines the importance of DNA repair studies in every patient with symptoms consistent with COFS syndrome.

**Severe hypophosphatasia due to mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene. J.**

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Hypophosphatasia is one rare lethal short-limbed dwarfism occurring in approximately one per 100,000 births. It is an inherited disorder characterized by defective bone mineralisation and deficiency of serum and tissue liver / bone / kidney alkaline phosphatase activity. We report the characterisation of tissue-nonspecific alkaline phosphatase (TNSALP) gene mutation in a patient affected by infantile hypophosphatasia. This boy was the first child of non affected, non related parents, mother 21 years old and father 30 years old. Pregnancy and delivery were normal. At 1 month of age appeared palsy of the left upper limb with hypotonia. Length 51 cm was 2SD, head circumference was 36,5 cm (- 1SD). Anterior fontanel was large. There was a markedly decreased ossification of all bones. All limbs were shortened. Ultrasonographic examination of the kidneys showed nephrocalcinosis. Level of alkaline phosphatases was decreased in the child at 20 U/l. as well as in the parents : mother 37 (N=55-195) and father 54 U/l. Bone density was decreased : Z score 6. At 2 years of age development was delayed. Weight was 3,5 SD and OFC 3SD. The child had craniosynostosis. Molecular studies showed a mutation R167W and a mutation E174K, both in exon 6 of the TNSALP gene. Both are missense mutations and were reported previously at least once in European patients but not together in the same patient. These mutations were not found in 40 and 57 individuals of the general population, respectively. The major cause of short-limbed dwarfism with hypomineralization of bones include hypophosphatasia, osteogenesis imperfecta type II and achondrogenesis. Hypophosphatasia has an autosomal recessive pattern of inheritance with a 25% chance of recurrence in the next pregnancy. Characterization of the mutation allows prenatal diagnosis and preimplantation diagnosis.

**OSTEOCRANIOSTENOSIS-Skull configuration mimicking kleeblattschadel with gracile long bones. Two new cases with distinctive chondro-osseous morphology.** *A.M. Elliott<sup>1</sup>, W.R. Wilcox<sup>1</sup>, G.S. Spear<sup>2</sup>, T.S. Steffensen<sup>3</sup>, D.L. Rimoin<sup>1</sup>, R.S. Lachman<sup>1</sup>.* 1) International Skeletal Dysplasia Registry, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) University of Irvine-Irvine, CA; 3) Melrose Wakefield Hospital, Melrose, MA.

We report two unrelated male infants with osteocraniostenosis and describe the clinical, radiographic and chondro-osseous morphology findings. Osteocraniostenosis is a skeletal dysplasia characterized by a skull configuration mimicking kleeblattschadel (cloverleaf skull) and overtubulated long bones. Dysmorphic features include small palpebral fissures, short nose and philtrum and a small, inverted v-shaped mouth. Splenic hypoplasia is a constant finding. Patient #1 died shortly after birth at 39 weeks gestation. Autopsy revealed a dysmorphic baby with length -4 SD, weight 3rd centile and OFC -3 SD. Radiographic examination showed a kleeblattschadel appearance, platyspondyly and saggital clefting mainly of the thoracic region of the spine. The long bones had moderate shortening with metaphyseal flaring and rounding. The diaphyses of the long bones and short tubular bones of the hands and feet were overtubulated. Histological examination of bone revealed an abnormal growth plate with short irregular columns. The resting cartilage showed pleomorphic chondrocytes with increased cellularity and unique pseudocolumn formation. Patient #2, born at 36.5 weeks gestation, died shortly after birth. Measurements included: length -4 SD, weight -4 SD and OFC 25th centile. Patient #2 resembled patient #1 clinically and radiographically. Both patients had hypoplastic cranial bones, eye abnormalities, pulmonary hypoplasia, splenic hypoplasia and hepatomegaly. The chondro-osseous morphology was similar in both patients. Although gracile bones are uncommon in the skeletal dysplasias, they are often associated with fetal hypokinesia. The bone histology is usually normal in those patients except for thin cortices with growth plate and diaphyseal fractures. Patients with Hallermann-Streiff syndrome (HSS) also have slender tubular bones. Radiographic and histological similarities between HSS and the two patients reported here are discussed.

**A new autosomal dominant syndrome with strabismus, cleft palate, patellar dislocation, and facial dysmorphic features.** *V.M. Der Kaloustian, F. Fassier, M. Flanders, R. Crépeau, G. Rouleau.* McGill University Health Ctr., McGill Univ, Montreal, Quebec, Canada.

We report a family with four persons affected with various combinations of strabismus, cleft palate, patellar dislocation, and facial dysmorphic features. The probanda is a 5-year-old girl, the second child of a non-consanguineous French-Canadian couple. She was born at term with Apgar scores of 9 and 10 at 1 and 5 minutes respectively. At birth she had a cleft palate and was operated for it at the age of one year. She has right superior oblique muscle palsy, narrow and upslanting palpebral fissures, micrognathia, and a particular triangular shape of the pinna with a horizontal upper border. She also had bilateral congenital patellar dislocation for which she was operated at the age of 3 and a half years. The family history is positive for congenital anomalies. The brother, who is 7 years old, also has superior oblique palsy, similar ear anomalies and micrognathia. The father has the same eye and ear anomalies, micrognathia, and had bilateral patellar dislocation for which he was operated at the age of 12 years. A paternal aunt had cleft palate and eye anomalies. We think that our patients are affected with a new syndrome following the autosomal dominant pattern of inheritance with variable expressivity.

**Orthopedic manifestations in neurofibromatosis type 1.** *S. Boero<sup>1</sup>, G. Stella<sup>1</sup>, D. Sambarino<sup>2</sup>, S. Massa<sup>2</sup>, S. Costabel<sup>2</sup>, L. Costabello<sup>2</sup>, C. Bellini<sup>2</sup>, E. Bonioli<sup>2</sup>.* 1) Orthopedic Department, G. Gaslini Children Institute, Genova, Italy; 2) Pediatrics Department, University of Genova, Italy.

Scoliosis is the most common orthopedic manifestation of NF1 as it is observed in 12-20% of patients. In some patients it is indistinguishable from idiopathic scoliosis and its greatest rate of curve progression occurs during the adolescent growth spurt; the specific NF1 types of scoliosis are associated with vertebral dysplasia, most commonly in the form of a short-segment angular thoracic lesion. Congenital bowing may involve any long bone, the tibia being the most commonly affected. It occurs in a small percentage of NF1 patients (3%) and usually evolves into pseudoarthrosis. We observed 34 NF1 patients with skeletal manifestations with a yearly follow up. 21 of these showed lumbar or thoracic scoliosis and 13 showed leg deformities. Seven cases were not surgically treated: 3 of these presented a mild not evolutive scoliosis; 2 young children presented a bow tibia, and were treated with a protective brace; 2 other cases with scoliosis were not operated because of serious respiratory difficulties. 16 patients with scoliosis have been treated with a Milwaukee brace and then with anterior and/or posterior fusion, using the Harrington or C.D. instrumentation. A good consolidation has been obtained in 14, while in 1 patient the vertebral fusion was eroded by a large dural ectasia that caused relapse of the deformity and paraplegia; 1 patient died following a mediastinum and brain stem neoplasia. 11 patients with pseudoarthrosis have been treated. Most of them underwent different procedures in different times: Ilizarov's technique, fibula-pro-tibia graft, infiltration of methylprednisolone acetate. Consolidation has been obtained in 9 cases, but the tibia in 2 of these has a residual shortening. In our experience, in case of dysplastic scoliosis better results have been obtained with antero-posterior arthrodesis; in case of bow tibia the later the Ilizarov's procedure is performed the better are the results in term of rate of consolidation and stability.

**Desbuquois syndrome: clinical, radiographic and long-term outcome in 15 cases.** *V. Cormier-Daire<sup>1</sup>, L. Faivre<sup>1</sup>, P. Maroteaux<sup>1</sup>, A. Munnich<sup>1</sup>, R. Lachman<sup>2</sup>, M. Le Merrer<sup>1</sup>.* 1) Dept Genetics, Hosp Necker, Paris, France; 2) Dept Pediatric Radiology, Cedars Sinai, Los Angeles, USA.

Desbuquois syndrome is a rare condition of autosomal recessive inheritance, characterized (i) clinically by markedly decreased birth length, facial dysmorphism and joint laxity, and (ii) radiologically by a Swedish key appearance of the proximal femur, coronal clefts of the spine and characteristic hands and feet abnormalities including delta phalanx and/or duplication of the thumb, extraossification center distal to the second metacarpal and advanced bone age. Here, we report on 15 patients (12 boys, 3 girls) with Desbuquois syndrome from 4 consanguineous and 10 non-consanguineous families, originating from 7 different countries. Birth length was less than 44 cm, facial dysmorphism and joint hyperlaxity were noted in all patients. Abnormal thoracic shape was noted in 11/15 patients, and mental retardation in 4/15 patients. Out of 15 cases, 4 patients died from age 2 months to 6 years of respiratory insufficiency. The mean age of the survivors was 5 years and their long-term outcome was characterized by severe growth deficiency, from 4 to 9 standard deviations, cyphoscoliosis and marked invalidity in the older patients. Swedish key appearance of the proximal femur, flat acetabular roof, short first metacarpal and metatarsal, advanced carpal and tarsal bone age, large and flat metaphyses, coronal and sagittal clefts of the spine, and midface hypoplasia were consistent radiological features in all patients. Radioulnar dislocation was found in 4/15 cases. It is worth noting that supranumerary ossification centers of the hands and feet, delta phalanx of the thumb and finger deviation and cleft thumb were found in 5/15 cases only, and polydactyly in 1/15 cases. We therefore suggest that hand abnormalities are not mandatory for the diagnosis of Desbuquois syndrome.



**Rhizomelic Limb Shortness in NF1: A Previously Unrecognised Skeletal Manifestation?** *L. Dupuis, A.S. Teebi, M.M. Nezarati.* Hosp Sick Children, Toronto, ON, Canada.

Distinctive osseous lesions may occur in individuals with Neurofibromatosis type 1 (NF1). Commonly, these lesions affect local bone growth. We report a case of a 22-year-old woman with a clinical and molecular diagnosis of NF1 as well as severe bilateral rhizomelic shortness of the upper limbs. The proband was born to a consanguineous Portuguese couple. As a child, she had "swellings" removed in the axillary regions and the lateral aspect of the right leg. Development was reported as normal and she completed high school with some difficulty in mathematics. Examination revealed nine café-au-lait spots (>1.5 cm in diameter) with no axillary or inguinal freckling or neurofibromas. Height was 153 cm and arm span was 134 cm. She had a long face with mild mid-face hypoplasia, a narrow palate and mild dental malocclusion. Ocular examination revealed multiple Lisch nodules. Skeletal findings included diaphyseal shortness and narrowing of humeri, more pronounced on the right. Cortical thickening was seen along the medial proximal diaphysis of both femurs. She had tibial bowing on the right but not true pseudoarthrosis. The left tibia was unremarkable. She had mild scoliosis in the lower lumbar region with one anteriorly scalloped vertebral body. Protein truncation analysis revealed premature termination of protein translation in the amplified cDNA fragment spanning NF1 exons 22-28. There was no family history of NF1. One of the seven siblings was reported to have one leg thinner and longer than the other. The patient recently delivered a son with no apparent features of NF1 and no limb abnormalities. The most common types of osseous lesions associated with NF1 include pseudoarthrosis, congenitally bowed tibia without pseudoarthrosis, scoliosis and dysplastic sphenoid wing. Bony changes secondary to plexiform neurofibromas have been well documented. Our patient's skeletal findings are unusual. The asymmetric shortness of the humeri are more likely due to primary skeletal involvement, rather than secondary effects of plexiform neurofibromas. This case further expands our understanding of skeletal manifestations associated with NF1.

**Spondylo-Meta-Epiphyseal Dysplasia, Short Limb-Abnormal Calcification Type: Clinical and Radiological features in 3 patients and Exclusion of the DTD gene.** Z. Ben-Neriah<sup>1</sup>, V. Cormier-Daire<sup>2</sup>, C. Huber<sup>2</sup>, J. Sosna<sup>3</sup>, J. Bar-Ziv<sup>3</sup>, A. Raas-Rothschild<sup>1</sup>. 1) Department of Human Genetics, Hadassah Hospital, Jerusalem, Israel; 2) INSERM U393 and Department of Genetics, Hospital Necker Enfants-Malades, Paris, France; 3) Department of Radiology, Hadassah Hospital, Jerusalem, Israel.

In 1993, Borochowitz reported on three patients with a new bone dysplasia which he called, Spondylo-Meta-Epiphyseal Dysplasia (SMED), Short Limb-Hand type [MIM, 271665]. Langer et al who proposed to call it SMED, Short Limb-Abnormal Calcification type, emphasized the importance of the chondral calcifications in the diagnosis of this disorder. SMED, Short Limb-Abnormal Calcification type (SMED, SL-AC) is an autosomal recessive condition with a grave prognosis. Spinal cord compression due to atlanto-axial instability and respiratory disease are the most common causes of death. Recently, we examined 3 patients up to the age of 16 years from 2 different consanguineous Arab Muslim families with this condition. We present the clinical and radiological findings that permit the diagnosis of SMED, SL-AC. Recently, Megarbane et al reported on a child with a variant diastrophic dysplasia and a new mutation in the DTD gene. The resemblance between this patient and our patients suggested a possible common etiology but sequencing of the entire coding sequence of the diastrophic dysplasia (DTD) gene did not reveal any mutation. Since the molecular defect of this disorder is still unknown, families at risk can be offered prenatal diagnosis by means of early ultrasonographic examination as it was done for two of the families reported.

**Microcephalic Osteodysplastic Dysplasia: Progression of radiographic manifestations not previously described.**

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Microcephalic Osteodysplastic Dysplasia (Saul-Wilson Dysplasia) is a rare condition with a characteristic facies (prominent forehead with a small appearing face, midface hypoplasia, prominent eyes, downslanted palpebral fissures, narrow nasal root, beaked nose, and micrognathia), microcephaly, cataracts, severe postnatal growth deficiency, brachydactyly, clubfoot deformities, syrinx of the spinal cord, and variable developmental delay. All reported cases have been sporadic. Radiographic manifestations in reported cases of this condition include: platyspondyly, irregularity of the vertebral bodies, odontoid and L1 hypoplasia, overtubulation of the long bones with widened metaphyses, coxa valga, short metacarpals, metatarsals, and phalanges with irregular metaphyses, coned and ivory phalangeal epiphyses, and accessory ossification centers in the metacarpals and metatarsals. We describe the striking progression of radiographic manifestations, from infancy to 13 years, including odontoid with cervical spine subluxation, precocious calcification (aorta and costochondral cartilage), progressive osteopenia, unusual paddle shaped ribs, hypoplasia of the ulnae, and bowing of the ulnae and radii, accompanying other dramatic skeletal findings. These unusual radiographic findings are described in a previously reported case of microcephalic osteodysplastic dysplasia (Hersh, et.al; *Am J Med Genet* 51:194-199, 1994).

**Deletion 13q and skeletal dysplasia in a mexican patient.** *J.A. Morales<sup>1,3</sup>, N.M. Macias<sup>1,3</sup>, L.E. Wong-Ley<sup>1,3</sup>, M.P. Gallegos-Arreola<sup>2</sup>, A.I. Vasquez<sup>1</sup>, L.E. Figuera<sup>1,3</sup>.* 1) Division de Genetica, CIBO-IMSS, Guadalajara, Mexico; 2) Division de Medicina Molecular, CIBO-IMSS, Guadalajara, Mexico; 3) Doctorado en Genetica Humana, CUCS-UdeG, Guadalajara, Mexico.

Deletion of 13q is an entity with a broad clinical spectrum, ranging from psychomotor delay to hand and foot anomalies. This deletion have been reported in six other cases; one with deletion comprising region q22-q31 and the others with deletion of 13q22-q32. We report the case of a 6-year-old male with intrauterine growth retardation, recurrent infectious events in the right knee and bilateral chronic middle-ear otitis. After clinical examination he showed moderate psychomotor delay, mild bilateral hypoacusia, general shortening of the metacarpals, metatarsals and phalanxes, predominantly in the distal phalanxes of the first and fifth fingers of all limbs and a karyotype anomaly of 46,XY, del(13)(q22q31). This case exhibited the characteristic phenotype of a skeletal dysplasia and an interstitial deletion of the long arm of chromosome 13. Given the clinical and radiological features, we believe that witin 13q22-q31 possibly lays a gene involved in bone development.

**Stuve-Wiedemann syndrome : two additional cases of prenatal diagnosis.** *J. Martinovic<sup>1</sup>, J. Tantau<sup>1</sup>, F. Encha-Razavi<sup>1</sup>, M. Vekemans<sup>1</sup>, J.M. Jouannic<sup>2</sup>, M.C. Aubry<sup>2</sup>, S. Lyonnet<sup>1</sup>, M. Le Merrer<sup>1</sup>, V. Cormier-Daire<sup>1</sup>.* 1) Dept de Genetique, Hopital Necker - Enfants Malades, Paris, France; 2) Service de Gynecologie et Obstetrique, Hopital Necker-Enfants Malades, Paris, France.

The Stuve-Wiedemann syndrome (SWS) is a rare skeletal dysplasia characterised radiologically by bowing of the long bones with internal cortical thickening and large metaphyses, and clinically by malignant hyperthermic episodes, respiratory insufficiency and early lethality in the majority of cases reported. Parental consanguinity and recurrence in sibs is highly suggestive of an autosomal recessive inheritance. No data are yet available on the gene locus of SWS. Here, we report on two cases of SWS in the second and third trimester fetuses issued from two different non-consanguineous kindreds. In both cases, shortened and bowed femora and tibiae with wide metaphyses were cardinal signs prenatally. The pregnancies were interrupted at 24 and 30 weeks respectively. Radiological postnatal analysis confirmed bowed femora, tibiae, and fibulae with cortical thickening and wide, striated metaphyses, generalised skeletal hypomineralisation, strongly suggesting in both cases the diagnosis of SWS. Bone histology of femoral upper metaphyses was highly abnormal and showed irregularity of the chondroosseous junction, thick trabeculae, osteoclastic hyperactivity but normal resting cartilage. To our knowledge, this is the second report on prenatal diagnosis of SWS. Further studies are necessary to shed light on the genetic basis and pathogenesis of SWS.

**A new form of familial autosomal recessive, chondrodysplasia punctata.** *A. Pai<sup>1</sup>, S. Viero<sup>1</sup>, M. Thomas<sup>1</sup>, D. Myles Reid<sup>1</sup>, A. Toi<sup>1</sup>, R. Lachman<sup>2</sup>, D. Chitayat<sup>1</sup>.* 1) Mount Sinai Hospital, Toronto, Canada; 2) The International Registry of Skeletal Dysplasia(4), LA, California, USA.

Chondrodysplasia punctata is a heterogenous condition associated with exposure to teratogens, chromosome abnormalities and single gene disorders. Rhizomelic chondrodysplasia punctata is in most cases the result of a peroxisomal disorder. We report a new autosomal recessive type of chondrodysplasia punctata with normal peroxisomal function. A 24-year old primigravida woman was referred to us at 20 weeks gestation with fetal ultrasound findings of short limbs (the BPD measurement was consistent with 21.4 wks, the abdominal circumference with 19.7 wks, the femur 16.9 wks, the tibia 17.4 wks, the humeri 17.1 wks and the ulna 17.3 wks gestation). The chest to abdomen ratio was 82%. The facial features were abnormal with a sloped forehead, depressed nasal bridge with flat nose, micrognathia and mid-facial hypoplasia. There were abnormal vertebral calcifications and decreased movements. The patient had a premature delivery at 22 weeks gestation. The autopsy showed a globular head with midface hypoplasia, flat nose with no nasal septum and creases on both alae nasi. The eyes were prominent with hypoplastic supraorbital ridges and retrognathia. The chest was narrow, the abdomen was prominent and the long bones showed rhizomelic shortening with limitation in extension of both elbows. Radiographic assessment showed stippled epiphyses, platyspondyly with rhizomelic short long bones, anterior bowing of the femurs and clinodactyly of the fifth fingers and toes. No brain or cardiac abnormalities were found and the karyotype was normal and female (46,XX). Peroxisomal functions (Dr. A. Mosers lab) were normal. The second pregnancy was with another partner and resulted in a normal daughter. The third pregnancy was with the first partner and resulted in a fetus with gastroschisis and the same skeletal findings and facial features as in the first pregnancy. The karyotype was normal and male (46,XY). Based on the above information, we believe this is a hitherto new autosomal recessive condition associated with rhizomelic chondrodysplasia punctata and typical facial features with normal peroxisomal functions.

**Classical Ehlers-Danlos syndrome in mother and daughter.** *S. Ritz, S. Sastry, M.G. Bialer.* Dept of Pediatrics, North Shore Univ Hosp/NYU School of Med, Manhasset, NY.

Ehlers-Danlos syndromes (EDS) are inherited disorders of connective tissue characterized by skin hyperextensibility, joint laxity and tissue fragility. The nosology of EDS subtypes was revised in 1997. Occasional reports of congenital heart disease in EDS have been dismissed as coincidental. We report a mother and daughter with classical EDS (type II or mitis type) and atrial septal defect (ASD). The mother is one of 7 affected individuals over 4 generations in an autosomal dominant pedigree. She walked at 19-20 mo. Development was otherwise normal. She began dislocating joints at 10 yr and describes about 15 dislocations of her left elbow, 4-5 dislocations of the left patella and dislocations of fingers and her right thumb which required surgery. At 31 yr, she has pains in many joints. She has easy bruising, skin hyperextensibility, scarring on her shins, and still has flexible fingers, ankles and patellae. She takes a long time to heal. She was diagnosed with ASD at 17 yr and required surgery at 24 yr. Her first daughter was unaffected, but her second daughter, at 6 mo, has significant joint laxity involving fingers, toes, wrists, ankles, patellae and hips. There are no obvious skin findings. An EKG done shortly after birth for heart rate of 240 revealed supraventricular tachycardia (SVT) and she was treated with Digoxin and Inderal. There was an aneurysm of the atrial septum with a small left-to-right shunt across the foramen ovale. Follow-up echocardiogram at 6 mo revealed a 10 mm secundum ASD. She has had no clinical recurrence of SVT, but remains on medication. There is a report of 2 sisters with EDS and VSD, one of whom also had ASD. All the other cases that we are aware of are singleton. Since ASD is multifactorial, concordance of ASD and EDS would be expected to occur 1 in 50 times if the 2 conditions are unrelated. On the other hand, since some cases of classical EDS have been shown to be due to mutations in type V collagen, which is also expressed in the atrioventricular valve of the developing mouse embryo, it is possible that some EDS-causing mutations may predispose to structural congenital heart disease.

**Clinical and molecular characterisation of Frontotemporal dementia linked to Human Chromosome 3.** *L. Chakrabarti and FReJA (Frontotemporal Research in Jutland Association).* MRC Prion Unit, Imperial College, London, UK.

We are studying a large multigenerational Danish kindred in which there is autosomal dominant pre-senile dementia. Our group assigned this disease to the pericentromeric region of chromosome 3. Age of onset is between 50 and 65 years. Typically this dementia begins with a personality change. Features that follow include disinhibition, hyperorality, restlessness and dyscalculia. There are progressive behavioural problems, speech decline, and stereotypical behaviour, some motor disturbances and dystonic posturing. There is a lack of patient insight. We reported anticipation as a feature of this disease and statistical tests supported this theory. We now have data from 5 paternal transmissions and 12 maternal transmissions. A decrease in age of onset was seen in cases of paternal transmission but not when disease was inherited maternally. Maternal inheritance coincided with a similar or slightly later age of onset. The results of performing a two sample t test with unequal variances (Welch's t test) showed that there are significant differences in age of onset. The child's age of onset was significantly younger ( $P < 0.0001$ ) for paternal transmission (mean=51.6, sd=1.82, n=5) than for maternal transmission (mean=60.75, sd=3.82, n=12). When calculating the child-parent age of onset the difference was significantly less ( $P = 0.001$ ) for Paternal transmission (mean=-8, sd=2, n=5) than for Maternal transmission (mean+3.17, sd=2.76, n=12). Two new cases were diagnosed in this family last year and though both cases (they are siblings) have inherited the disease through the paternal line, they each have a later age of onset than their father. We have designed a program that can identify di, tri, tetra and penta repeats from genomic sequence and are testing these repeats to see if any are expanded in affected individuals. We are sequencing genes in the region to find a disease causing mutation. Genealogical studies are progressing in order to identify further informative meioses. Expression studies are underway using microarray technology to compare brain tissue from this family with matched tissue from Alzheimer's and non-neurodegenerative brain.



**Congenital hydrocephalus, ambiguous genitalia in males and early lethality: a new syndrome?** *L. Basel-Vanagaite*<sup>1</sup>, *G. Klinger*<sup>2</sup>, *M. Shohat*<sup>1,3</sup>, *Y. Udler*<sup>1</sup>, *O. Levit*<sup>2</sup>, *N. Linder*<sup>2</sup>, *L. Sirota*<sup>2</sup>. 1) Medical Genetics, Rabin Medical Center, Petah Tikva, Israel; 2) Department of Neonatology, Schneider Children's Medical Center of Israel, Petah Tikva, Israel; 3) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Congenital hydrocephalus can be caused by environmental and genetic factors. It can be an isolated abnormality or be a part of a syndrome. Further heterogeneity also exists among isolated cases of hydrocephalus. The X-linked form is caused by L1CAM mutations, but rarely it can be inherited as an autosomal recessive condition. We report on three infants, 2 male and 1 female, from two unrelated consanguineous Arab families living in the same village, with a previously undescribed pattern of malformations including congenital hydrocephalus, ambiguous genitalia in males, and early lethality. There are in addition five healthy children between the two families. Physical examination of the patients revealed severe hydrocephalus, low set ears and simian lines. Genital examination revealed normal genitalia in the female, but the males had micropenis with cryptorchidism and bifid scrotum. Metabolic and endocrine studies were normal. All the patients had normal karyotypes. CT scan revealed extreme dilatation of the lateral and the third ventricles but a normal fourth ventricle. Echocardiography in one of the male infants demonstrated double outlet right ventricle. All of the patients died at age 1 to 40 days despite insertion of ventriculoperitoneal shunts. In addition, each of two previous pregnancies in one of the families ended at 30-31 weeks of gestation with antepartum fetal death. Both fetuses had severe hydrocephalus. The patients in these two families seem to suffer from the same disease, inherited in an autosomal recessive pattern. The congenital heart disease in one of the patients could be a coincidental finding and not part of the syndrome, as it was not present in the other affected children. We suggest that this combination of anomalies constitutes a unique syndrome.

**"Apple-Peel" Intestinal Atresia, Ocular Anomalies and Microcephaly Syndrome. Brain Magnetic Resonance Imaging Study.** *C. Arioni, M. Mazzella, M.P. Fondelli, D. Sambarino, S. Massa, E. Bonioli, C. Bellini, G. Serra.*

Department of Pediatrics , Gaslini Institute, Genoa, Italy.

The patient was the first son of healthy, unrelated, normocephalic parents. At the 28th week of intrauterine life US scanning detected intestinal ectasia, suggesting small bowel obstruction. His birth weight (37 week pregnancy) was 1890 g and birth length was 44 cm (both at 10th centile), and head circumference was 27 cm (below the 3rd centile). X-ray examination at birth showed a intestinal ectasia. Barium roentgenogram confirmed upper jejunal obstruction. High jejunal atresia with apple-peel deformity and colonic malrotation was surgically identified. Ocular examination showed bilateral corneal leukoma which was more evident in the left eye. Fundoscopy was not possible due to corneal clouding. Ultrasound examination showed normal retina and optic nerve. MR brain examination (age two months) showed a microcephalic brain with normal brain substance and myelination pattern (neither malformations nor hydrocephalus). No ocular abnormalities were found. High resolution karyotype was normal. TORCH complex was negative. Urine culture for Cytomegalovirus was also negative. Spinal fluid analysis ruled out an infection. Association of apple-peel atresia with microcephaly and ocular anomalies has been reported only 3 times. We report on a fourth infant and the first brain MRI study in this syndrome. The absence of abnormalities of migration might be compatible with the absence of alcoholism, malnutrition, and diabetes mellitus in the mother. In this view, a genetic origin of microcephaly rather than a disruptive one is likely in our patient. Two out of the three previously reported patients have been found to be mentally retarded. Our report confirms that the association of apple-peel atresia, ocular abnormalities, and microcephaly is a distinct syndromic entity. Brain MRI evaluation should be included in the investigation protocol of such patients since it could provide important information regarding pathogenesis, genetic counseling, and patient management.

**Persistence of Mullerian Derivatives and Intestinal Lymphangiectasis in Two Newborn Brothers.** *C. Bellini<sup>1</sup>, M. Mazzella<sup>1</sup>, E. Bonioli<sup>1</sup>, C.M. Hennekam<sup>2</sup>, A.R. Sementa<sup>3</sup>, C.E. Marino<sup>3</sup>, D. Sambarino<sup>1</sup>, S. Massa<sup>1</sup>, G. Serra<sup>1</sup>.* 1) Department of Pediatrics, Gaslini Institute, Genoa, Italy; 2) Department of Pediatrics and Clinical Genetics Academic Medical Center, Amsterdam, The Netherlands; 3) Servizio di Anatomia Patologia, Istituto G. Gaslini, Genova, Italia.

The proband (case 1) was the liveborn male product of a third pregnancy (38 weeks). At birth, generalized lymphedema was immediately evident. There was a broad nasal bridge, a bulbous nasal tip, a long and prominent upper lip with smooth philtrum, and mild retrognathia, a thick alveolar ridges. The testes had descended properly, and the penis was normally formed. The presence of a uterus and the upper part of a hypoplastic vagina was US and MRI demonstrated. The anti-Mullerian hormone level was 76 ng/ml (normal). Karyotype was 46, XY; VLCF Acid and Plasmalogen, cholesterol metabolism were normal. Intestinal lymphangiectasis was demonstrated. He died of multiorgan failure caused by septic shock (three months). Autopsy showed generalized intestinal and pulmonary lymphangiectasia, a rudimentary uterus, Fallopian tubes, the upper third of a vagina, and a prostate of normal shape. The testes were situated in the scrotum. The brother's clinical course was identical. We describe two newborn brothers with a pattern of malformations characterized by the persistence of Mullerian duct derivatives (PMD) and intestinal lymphangiectasia. The syndrome was first delineated by Urioste (AJMD,47,494,1993). Our cases confirm the existence of a definite and separate entity. All patients died at a very young age. The hypogammaglobulinemia and lymphopenia can easily account for sepsis that can ensue in fatal septic shock. It is still uncertain whether this entity is compatible with longer survival. All cases reported thus far have been males and their parents were all healthy and non-consanguineous. The autosomal recessive pattern seems to be the most probable model of inheritance. An X-linked recessive pattern is also possible, but it is likely that the presence of Mullerian duct derivatives will create a bias for the recognition of the entity in males, and causes the distorted sex ratio.

**Distinguishing features for schizophrenia on MRI brain scans of 22qDS adults.** *E. Chow*<sup>1,2</sup>, *D.J. Mikulis*<sup>3</sup>, *R.B. Zipursky*<sup>1,2</sup>, *A.S. Bassett*<sup>1,2</sup>. 1) Schizophrenia Research Program, Queen St. Site, CAMH, Toronto, ON, CANADA; 2) Department of Psychiatry, Univ of Toronto, ON, CANADA; 3) Department of Medical Imaging, Univ of Toronto, ON, CANADA.

**Background:** Up to 25% of adults with 22q11 Deletion Syndrome (22qDS) may develop a psychotic disorder such as schizophrenia (SZ). We have previously reported high frequencies of certain features (e.g. bright foci, cavum vergae, ventricular enlargement, cortical atrophy, skull base abnormalities) that are clinically detectable on brain and neck magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) scans in adults with 22qDS and SZ. However, it is unclear whether these qualitative MRI features are found in similar frequencies in 22qDS adults without a psychotic illness. **Objective:** To compare the frequencies of MRI features between 22qDS adults with SZ and those without psychosis. **Method:** Brain and neck MRI and MRA scans of 24 adults with 22qDS (16 with SZ and 8 non-psychotic) were reviewed blind to psychiatric status by a neuroradiologist (DJM) for detectable cavum vergae/septum pellucidum, ventricular enlargement, cortical atrophy, bright foci, skull base abnormalities, and vessel anomalies. Frequencies of these features were compared between the two groups using Fischer's exact test. **Results:** More cavum anomalies (50% vs 0%,  $p=0.05$ ) and ventricular enlargement (50% vs 0%,  $p=0.05$ ) were noted in 22qDS subjects with SZ than those without psychosis, although the two groups had no significant difference in rates of bright foci, vessel anomalies, and skull base anomalies. **Conclusions:** Although sample size was small, results suggest that midline defects and ventricular enlargement detectable on clinical readings of brain MRI may be distinguishing features between 22qDS patients with and without SZ.

**Prenatal cerebral vasculopathy with vascular accident in an infant with neurofibromatosis 1.** *K.A. Fjelstad, J.J. Mulvihill, D.A. Wilson.* Children's Hospital of Oklahoma, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

The central nervous system features of neurofibromatosis 1 (NF1) are usually neoplastic, but reports of vascular abnormalities abound. A two-month-old infant was admitted in status epilepticus. The infant was afebrile and had no current illness. He had no prior seizures and a normal birth history. His mother, brother, and four other relatives had NF1 in five generations without life-threatening complications. The infant had mild left hemiparesis after cessation of seizure activity, macrocephaly, over six cafe-au-lait spots, and a right bony temporal mass with central fluctuance. Computerized tomography and magnetic resonance imaging revealed a right sided arachnoid cyst eroding bone. Evolving infarction of the right middle and posterior cerebral artery distributions, an atrophic right hemisphere, a lesser degree of left sided atrophy of the anteriortemporal lobe, and right basal ganglia hyperdensities were present. Magnetic resonance angiography displayed a one centimeter flow gap in the right middle cerebral artery and beading with multiple areas of stenosis in the right posterior cerebral artery. The right anterior cerebral, anterior communicating, and posterior communicating arteries were absent. The left-sided cerebral vasculature was normal. Cerebral vascular accident and NF1 were diagnosed in this infant. A history of chronic ischemia and likely in utero infarction were suggested by the cerebral atrophy. This case, perhaps the first with diffuse unilateral cerebral vasculopathy and arachnoid cyst in an infant with NF1, demonstrates the extreme variability in expression of NF1 even among members of the same family. It points to the prenatal origins of the vasculopathies of NF1, although clinical presentation and recognition are usually at an older age than this patient. In an infant at risk of NF1, head asymmetry might be sufficient indication for imaging studies; prenatal imaging might also detect such vascular defects.

**Central Auditory Processing (CAP) in Huntington's Disease (HD).** *T.S. Bradham<sup>1</sup>, R.K. Abramson<sup>2</sup>, E.M. Frank<sup>3</sup>, W.A. Cooper<sup>3</sup>.* 1) Medical Univ South Carolina, Charleston, SC; 2) W.S. Hall Psych. Inst, Columbia, SC; 3) Univ. South Carolina, Columbia, SC.

The purpose of this study is to determine if HD patients (HD) and presymptomatic gene positive persons (PreHD) show impaired CAP abilities as compared to matched controls. If dysfunction is present, this may suggest why persons with HD have difficulties with components of receptive and expressive language. Little is known about CAP dysfunction in HD. Impaired language function in HD, shown by a decrease in number of words used in connected speech, word finding difficulty, and loss of prosody in conversational speech (Yorkston et al, 1995) is also seen in people with CAP deficits. Anatomic and functional evidence implicate CAP involvement in HD. Two major auditory pathways ascend through the basal ganglia in the cerebral cortex. Secondary effects could occur in HD due to changes in the striatum, lateral ventricles, and decrease in overall brain weight (Lenz and Musiek, 1996). There have been no studies involving behavioral tests of CAP function. Twelve HD and 8 PreHD participants were matched for age, education level, and gender with people with no known neurological disorders. Participants (n=40) had peripheral hearing and CAP evaluations. Peripheral hearing assessments included tympanometry, acoustic reflexes, pure tone and speech audiometry, and transient evoked otoacoustic emissions. The CAP test battery included synthetic sentence identification with ipsilateral competing message, dichotic digits, and duration pattern test. Data were analyzed using analysis of variance. Peripheral auditory functions were not significantly different between groups. HD participants scored significantly lower on the speech recognition test in each ear ( $p < .002$ ) compared to controls and on seven of the nine measures from the CAP test battery ( $p < .01$ ), indicating deficits in auditory figure ground, dichotic listening, and temporal sequencing. The PreHD group scored the same as the controls on the CAP test battery. Research is needed in this area. Testing for CAP dysfunction in HD would aid in decisions about placement, level of care, assistive technology and acoustical environment management.

**Mesial temporal lobe abnormalities in a family with 15q26qter trisomy.** *D. Facchin<sup>1</sup>, E. Kobayashi<sup>2</sup>, F. Cendes<sup>2</sup>, C.E. Steiner<sup>1</sup>, A.A.A. Leone<sup>2</sup>, N.L.V. Campos<sup>1</sup>, I. Lopes-Cendes<sup>1</sup>.* 1) Department of Medical Genetics, University of Campinas, Campinas, SP, BRAZIL; 2) Department of Neurology, University of Campinas, Campinas, SP, BRAZIL.

**Objective:** To describe the clinical, neuro-imaging and cytogenetic findings of two brothers with secondary generalized epilepsy (SGE) and mental retardation (MR).

**Methods:** Seizures and epilepsy syndrome, as well as the development milestones, were characterized by detailed clinical history. We performed neurologic examination, including cognitive evaluation with estimated IQ in both patients and all available relatives. After informed consent, we collected blood samples for cytogenetic studies on short-term culture of peripheral blood lymphocytes using standard G-banding technique. In addition, we performed routine interictal EEGs and MRI scans. Imaging studies were conducted in a 2Tesla scanner including T1 and T2 weighted images in three orthogonal planes with 3-6 mm slices. In addition, a T1 volumetric acquisition with 1mm thickness was performed. MRI analyses included detailed visual evaluation including multiplanar reconstruction.

**Results:** We report a family with two brothers presenting with 15q trisomy due to a maternal equilibrated translocation involving chromosomes 12 and 15. Clinical findings include minor dysmorphic features, mental retardation, abnormal behavior, and secondary generalized epilepsy. EEG showed left temporal slow waves in the oldest brother and background abnormality associated to generalized and multifocal epileptiform discharges in the other. Their MRI showed mesial temporal lobe malformation, including the hippocampus, parahippocampal gyrus and fusiform gyrus, with abnormal shape and axis.

**Conclusion:** To our knowledge, this is the first report of mesial temporal lobe malformation associated with chromosomal abnormalities. Our findings may contribute to the understanding of the genetic mechanisms involved in malformations of central nervous system, especially in the mesial temporal lobe structures.

**Psychosis and Ataxia in a Patient with a novel 46,XX, t(1;22)(p36.3;q11.2) translocation.** *T. Causey<sup>1</sup>, R.K.*

*Abramson<sup>2</sup>, T. Malone<sup>1</sup>, R.G. Best<sup>1</sup>.* 1) USC School Medicine, Columbia, SC; 2) W.S. Hall Psych. Inst., Columbia, SC.

A 16 yo white female was seen for withdrawal, anxiety, and bizarre jerking episodes. Physical exam revealed normal height, weight, relatively square nasal root, prominent tubular nose with bulbous nasal tip and hypernasal speech. Her IQ was 63 with problems in arithmetic, attention span, letter number sequence, matrix reasoning and symbol search. General and verbal memory, attention/concentration and delayed recall were impaired. Thyroid, B12, folate, bHCG, and ceruloplasmin were normal. Psychiatric evaluation revealed constricted, anxious and paranoid affect. She was mute, and had auditory and visual hallucinations and suicidal ideation. Neurologic evaluation to rule out seizures revealed ataxic gait, spastic paraparesis, and a history of "passing out spells". MRI and EEG were unremarkable, but the neurologist noted that the proband is at risk for a seizure disorder. Cytogenetic studies indicated a 46,XX,t(1;22)(p36.3;q11.2) translocation. The patient has features of Velocardiofacial Syndrome (VCFS) at 22q11.2 (physical features, blunt affect, psychosis and learning disability). A potassium voltage channel gene, KCNB2 is present at 1p36.3. There are no reports in the literature of a syndrome associated with this gene. However, a related gene, KCNA1 on chromosome 12 is associated with periodic ataxia with onset in the second decade, and myokymic discharges, demonstrable on electromyography but not always clinically detectable. Other potassium voltage channel genes have been associated with epilepsy. Family studies indicate that the father has the same translocation. FISH analysis for VCFS deletion was negative for both father and proband, but about 5% of these microdeletions are not detectable by this method. The father reports no psychosis or seizures, and works for a steel company. His affect was rather flat. He has one sister who did not complete high school, self isolates and has seizures. There is no maternal or paternal history of psychosis. Further studies on the proband and the extended family are in progress to better define the translocation and the clinical findings in this very interesting family.



**Thin anterior corpus callosum, dementia, spastic paraparesis and leukoencephalopathy: a distinct autosomal recessive disorder.** *G.M. Bibat<sup>1</sup>, F.M. Murillo<sup>2</sup>, E.R. Melhem<sup>3</sup>, H.W. Moser<sup>1</sup>, S. Naidu<sup>1</sup>.* 1) Div Neurology & Dev Medicine, Neurogenetics Unit, Kennedy Krieger Inst, Johns Hopkins University School of Medicine, Baltimore, MD; 2) Department of Molecular Genetics and Biochemistry, Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 3) Department of Neuroradiology, Johns Hopkins Hospital, Baltimore, MD.

We report 7 males (3 of whom are brothers) from 5 unrelated families aged 3 to 31 years with thin anterior corpus callosum, periventricular white matter abnormalities, slowly progressive dementia and spastic paraparesis. Onset of symptoms range from birth to 25 years of age. Three of the 7 who were evaluated early in life were noted to be hypotonic and developmentally delayed. The adults, including 3 brothers and an unrelated patient, presented with early learning difficulties requiring special education. Gait worsened from the second decade of life. Progressive spasticity became prominent with age. However, all 7 had early cognitive impairment, speech difficulties and varying degrees of behavioral issues. The 3 brothers have a female sibling who is reported to have similar clinical manifestations as her brothers. Peripheral neuropathy with axonal degeneration was seen in the older males. Neuroimaging showed periventricular leukoencephalopathy and non-progressive thinning of the corpus callosum. Medial frontal atrophy was noted in those 20 years and older suggestive of progression. Screening for metabolic, lysosomal and peroxisomal disorders was unremarkable. No mutations were found in the genes that code for paraplegin, PLP and L1CAM while screening for mutations in the spastin gene is in progress. An autosomal recessive form of spastic paraparesis with varying degrees of dementia and thin corpus callosum was localized to 15q13-15. Our patients differ from this group because the dementing illness, periventricular leukoencephalopathy and consistently thin anterior corpus callosum are more prominent. In most of our families, linkage to the 15qFSP locus was excluded. These patients form a unique entity with autosomal recessive mode of inheritance, early onset of mental retardation, progressive spasticity with a distinctive MRI picture.

**Macrocephaly, macrosomia and autistic behavior due to a de novo PTEN germline mutation.** *M.J. Dasouki<sup>1</sup>, H. Ishmael<sup>1</sup>, C. Eng<sup>2</sup>.* 1) Sect Medical Gen & Molec Med, Children's Mercy Hosp, Kansas City, MO; 2) Clinical Cancer Genetics and Human Cancer Genetics Programs, Ohio State University, Columbus, OH.

Bannayan Riley Ruvalcaba (BRR) is a dominantly inherited cancer syndrome characterized by the classic triad of macrocephaly, lipomatosis and pigmented macules of the glans penis. Other BRR component features include macrocephaly, lipomatosis, haemangiomas, Hashimoto's thyroiditis, mental and developmental delay. BRR syndrome is due to germline PTEN mutations. PTEN is a recently discovered tumor suppressor gene which appears to negatively control the phosphoinositide 3-kinase signaling pathway for regulation of cell proliferation and cell survival by dephosphorylating PIP3. Autism is a neuro- developmental disorder characterized by impairments in reciprocal social interaction and communication, restricted and stereotyped patterns of interests and activities. Some autistic children are also macrocephalic. While several chromosomal and metabolic disorders cause autism, many cases are still idiopathic. A 4 year old boy presented severe speech delay, autistic like behavior, macrocephaly and macrosomia. Other features of BRRS were lacking. His father was macrocephalic and an older brother has trisomy 21. Biochemical studies including urine organic acids, acylcarnitines, head MRI and bone survey were all unremarkable. Sequencing of the PTEN gene in the proband and his parents showed a de novo, novel germline mutation in codon 93, exon 4 [CAT>CTG]. Therefore, autism in association with macrocephaly and macrosomia may suggest the possibility of a PTEN mutation. Similar patients need to be examined to determine the frequency of PTEN mutations in this selected population. Also, new insights into the function of the PTEN gene may ultimately lead to a better understanding of the pathogenesis and treatment of this form of autism.

Board #565 will now be used by Marion Gerard-Blanluet to present a poster titled **Association of agnathia and craniorachischisis in a consanguineous family.**

Program Nr: 565

**Unspecific Mental Retardation: a Critical Diagnosis.** *G.S. Oliveira, A.P. Marques-de-Faria.* Dept Genetica Medica - FCM, UNICAMP, Campinas, SP, Brazil.

Mental retardation is one of the most common complaints in the Clinical Genetics' daily practice. It is very important to determine its etiology, as for a right and specific genetic counseling, as for a better patient evaluation and follow up. During a period of 5 years, we evaluated 3,245 patients in our outpatient clinic. From these, 316 individuals had Down syndrome (9.7%), 157 had other chromosomal aberration (4.8%), and 280 had mental retardation in several degree (8.6%). Within them 5 patients had X-linked mental retardation (1.8%), 68 had the diagnosis of autism (24.3%), and 212 patients had the diagnosis of mental retardation of unknown etiology (75.7%). All of these individuals did not have any relevant history of familial, gestational, neonatal, and personal data. Most of them had normal investigation, including karyotype with fra(X) negative, metabolic screening, skeletal X-ray, and CT brain scan, among others. None of them had major neither minor dismorphism that could lead to a specific clinical condition. Most of the patients are still being followed-up by our team. We consider important the continuous evaluation of these individuals, based upon the advance of new technologies for a specific diagnosis, specially to benefit the family with counseling and recurrence risks, and the patient with the correct management, avoiding stigmatization and labeling.

**A new X-linked lissencephaly syndrome with agenesis of corpus callosum: a gene locus on the Xpter-p22. K.**

*OBATA<sup>1</sup>, T. Fukuda<sup>1</sup>, J. Tohyama<sup>2</sup>, H. Yamagata<sup>1</sup>, R. Morishita<sup>1</sup>, I. Kondo<sup>1</sup>.* 1) Hygiene, Ehime University School of Med, Onsen-gun, Ehime, Japan; 2) Department of Pediatrics, Okinawa Child Development Center, Okinawa, Japan.

Two clinical syndromes with the lissencephaly and agenesis of the corpus callosum have been reported, an X-linked lissencephaly with ambiguous genitalia and an autosomal recessive lissencephaly with neonatal death. We report here two male infants with lissencephaly and agenesis of the corpus callosum born to non-consanguineous parents. The clinical features in these patients were characterized by severe growth failure, psychomotor retardation and intractable seizures with the neonatal onset. They had normal male type of external genitalia and testes descended into normal position. No microdeletion of 17p13.3 was detected by FISH analysis. No DNA mutation was identified in the doublecortin gene(DCX1) in both patients and their mother by DNA sequencing. Based on clinical characteristics and a mode of inheritance, our family might have a new X-linked lissencephaly syndrome with agenesis of the corpus callosum. The genetic locus for this disease has been studied using polymorphic DNA markers covering the whole X chromosome in three generations of this family. Two affected males had the same alleles of DNA markers on Xpter-p22 derived from their maternal grandfather, suggesting that the gene locus is likely to be mapped to Xpter-p22.

**Cornelia de Lange syndrome: evidence for a sensory neuropathy.** *A.D. Kline<sup>1</sup>, I. Krantz<sup>2</sup>, A. Goldstein<sup>3</sup>, B. Koo<sup>3</sup>, L.G. Jackson<sup>4</sup>.* 1) Harvey Institute for Human Genetics, Greater Baltimore Medical Ctr, Baltimore, MD; 2) Division of Human Genetics & Molecular Biology, Children's Hosp of Philadelphia, Philadelphia, PA; 3) Dept of Pediatric Neurology, Children's Hosp of Pittsburgh, Pittsburgh, PA; 4) Division of Medical Genetics, Jefferson Medical College, Philadelphia, PA.

Cornelia de Lange syndrome (CdLS) is a well-known syndrome characterized by typical facial features, small stature, limb findings, neurodevelopmental abnormalities and gastrointestinal involvement. Multiple affected individuals were observed to have features typically present in the sensory and autonomic neuropathies, such as decreased tears, sensory disturbances with abnormal pain and temperature sensations, the Raynaud phenomenon and gait abnormalities.

Individuals with CdLS may also have acute episodes apparently related to pain or discomfort with no detectable cause. Because of this, a possible link between CdLS and the sensory and autonomic neuropathies was postulated.

Questionnaire data were collected from 26 individuals with CdLS. Results include: 86% with a high tolerance or delayed reaction to pain; 60% with some inexplicable blotching of skin; 56% unable to tolerate cold; 47% unable to tolerate heat; 53% with absent or unusual sweating; 48% with decreased tears; 12% with absent tears; 47% with cold extremities; and 43% with some symptoms similar to Raynaud phenomenon. 24 individuals underwent a screening intradermal histamine testing; one was positive. EMG and nerve conduction velocities demonstrated a mild peripheral neuropathy. During sensory evoked potentials, the sympathetic skin response was abnormal, which demonstrated abnormal autonomic function. The modified tilt table and other neurologic testing to further evaluate autonomic function are pending. Based on preliminary results, there is evidence for autonomic dysfunction in CdLS. Identification of an association between CdLS and sensory and autonomic neuropathies could potentially help with gene localization for CdLS, and provision of possible treatment options.

**A new autosomal recessive syndrome of profound microcephaly.** *G.H. Mochida<sup>1</sup>, A. Rajab<sup>2</sup>, A. Bodell<sup>1</sup>, C.A. Walsh<sup>1</sup>.* 1) Neurology, Beth Israel Deaconess Medical Center, Boston, MA; 2) Genetic Unit, DGHA, Ministry of Health, Sultanate of Oman.

We report 4 Omani children from 3 consanguineous families with a new syndrome of profound autosomal recessive (AR) microcephaly. These patients are characterized by 1] congenital microcephaly, which is more severe than most reported cases with AR microcephaly, 2] cognitive delay with preserved alertness, 3] mild to moderate gross motor delay, and 4] an absence of seizures. Patient 1 is a 9 year-old boy with an uncomplicated prenatal and perinatal history. He walked at age 3, and currently speaks several words. Occipito-frontal circumference (OFC) at age 8 was 39cm (-10.0SD). Brain MRI showed a mildly simplified gyral pattern, a normal thickness of the cortex, and no other major structural abnormalities. Patient 2 is a younger brother of Patient 1, and is 2 years old. He is alert and attentive, but still unable to walk and has not spoken yet. Current OFC is 37cm (-9.0SD). Patient 3 is a 5 month-old boy, whose family is distantly related to the family of Patient 1 and 2. OFC at birth was 27.5cm (-6.3SD). He is alert and has a normal neurological examination, except for a mild increase in muscle tone. Current OFC is 32cm (-7.6SD). Patient 4 is a 5 year-old boy, who is from a tribe related to that of other three patients. OFC at birth was 29cm (-5.3SD). He walked at age 2, and cannot speak. Current OFC is 37cm (-10.1SD). Microsatellite marker analysis of the family of Patient 1 and 2 has ruled out linkage to known AR microcephaly loci, *MCPHI* through 5. Patients linked to *MCPHI* through 5 generally have microcephaly of -5 to -9SD; however, the patients reported here show microcephaly of -9SD or more, except for Patient 3, who is still only 5 months old. This severe degree of microcephaly, and the presence of gross motor delay, may clinically distinguish these patients from AR microcephaly with linkage to *MCPHI* through 5. The lack of linkage to known AR microcephaly loci further supports the presence of a novel microcephaly locus that has not been mapped. GHM is a Howard Hughes Medical Institute Physician Postdoctoral Fellow. Supported by the March of Dimes and the NINDS.

**Positive family history for epilepsy anticipates age of first seizure in patients with focal cortical dysplasia.** *M.A. Montenegro<sup>1</sup>, F. Cendes<sup>1</sup>, L.M. Li<sup>1</sup>, C.A.M. Guerreiro<sup>1</sup>, I. Lopes-Cendes<sup>2</sup>, M.M. Guerreiro<sup>1</sup>.* 1) Department of Neurology, University of Campinas, Campinas, SP, BRAZIL; 2) Department of Medical Genetics, University of Campinas, Campinas, SP, BRAZIL.

**Rationale:** Focal cortical dysplasia (FCD) is a malformation of cortical development characterized by abnormal cortical lamination and dysmorphic neurons. It is diagnosed by MRI as areas of cortical thickening, abnormal gyration, blurring between gray and white matter and focal atrophy. The age of first seizure in patients with FCD depends on many factors, such as size, localization and type of lesion. To our knowledge, no familial cases of FCD have been reported.

**Objective:** To analyze the influence of genetic predisposition in the age of first seizure in patients with FCD.

**Methods:** We evaluated 19 consecutive patients with FCD diagnosed by MRI. All patients were examined and interviewed by at least one of us. Detailed pedigrees were constructed for each family. In addition, we investigated family history of seizures and/or epilepsy in the families of all patients studied. Student's t test was used to perform the statistical analysis.

**Results:** Ages ranged from 3 to 41 years (mean=10.1). The mean age of first seizure in patients with positive family history of epilepsy was 2.6 years, as opposed to 8.5 years when there were no relatives presenting with epilepsy ( $p<0.05$ ).

**Conclusion:** To date, the contribution of genetic and environmental factors in the etiology of FCD remains unclear. We have contributed to this discussion by showing that a positive family history of epilepsy anticipates the age of first seizure in patients with FCD. Thus, suggesting that clinical presentation in these patients is influenced by genetic predisposition.

**Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB): Initial Clinical Characterization of the PI12<sub>Syracuse</sub> (S49P) Mutation.** *F.L. Lachawan<sup>1,5</sup>, R.L. Davis<sup>2</sup>, G.H. Collins<sup>2</sup>, P. Kent<sup>4</sup>, P.D. Holohan<sup>3</sup>, A.E.*

*Shrimpton<sup>2</sup>, M. Muenke<sup>5</sup>, D. Krasnewich<sup>5</sup>.* 1) Dept Medical Genetics, Children's Natl Medical Ctr, Washington, DC; 2) Dept of Pathology, SUNY Upstate University, Syracuse, NY; 3) Dept of Pharmacology, SUNY Upstate University, Syracuse, NY; 4) Dept of Neurology, SUNY Upstate University, Syracuse, NY; 5) Medical Genetics Branch, national Human Genome Research Institute, NIH, Bethesda, MD.

FENIB is a recently described autosomal dominant neurodegenerative disorder with polymers of neuroserpin (Collins bodies) in the gray matter cortical neurons and in certain subcortical nuclei that clinically presents as early-onset dementia. We report, based on review of medical records, the natural history of three deceased and two living affected individuals in the large family with the PI12<sub>Syracuse</sub> (S49P) mutation. The onset of overt clinical signs is between 45-52 years of age and may be aggravated by loss of spouse or job. They have confusion, flighty talk, communication difficulties with decreased verbal productivity, perseveration in task and thought processing, poor insight and impaired judgement. Gross memory loss, remote more than recent, poor calculation and visuo-spatial difficulties are evident but reading skills are intact. Stereotypic body movements, especially involving the extremities, include jerks, shakes, twitches or tremors. Although seizures can occur later in the course of the disorder, EEG studies usually showed diffuse slowing with disorganized background. CT scan demonstrated cortical atrophy in the living affected individuals. Working diagnoses included presenile dementia-probably Alzheimer type, severe dementia, chronic chorea and dementia, early subcortical dementia-rule out Huntington Disease and fronto-parietal lobe dysfunction, and tardive dyskinesia. The deteriorating clinical course of affected individuals runs from 10 to more than 20 years and the cause of death for the 3 members were complications of severe coronary artery disease, thromboembolism and vehicular accident. An ongoing prospective clinical study at the NHGRI-NIH is aimed at delineating the clinical manifestations of FENIB to facilitate its definitive diagnosis.



**Behavioral Phenotype of Nail-Patella Syndrome.** *I. McIntosh*<sup>1</sup>, *E. Tierney*<sup>2, 3</sup>, *I. Bukelis*<sup>2</sup>, *L. Marsh*<sup>3</sup>. 1) Inst Genetic Medicine, Johns Hopkins Medical Sch, Baltimore, MD; 2) Kennedy-Krieger Inst., Baltimore, MD; 3) Dept. Psychiatry, Johns Hopkins Medical Sch, Baltimore, MD.

Nail Patella Syndrome (NPS)(MIM 161200), is an autosomal dominant disorder that presents with dysplasia of the nails and absent or hypoplastic patellae. NPS is the result of loss-of-function mutations in *LMX1B*, a LIM-HD transcription factor essential for patterning of the developing limbs. Recently, *Lmx1b* has been shown to be expressed in mesencephalic dopaminergic neurons with high expression in the substantia nigra pars compacta and ventral tegmental area. Post-mortem studies of the substantia nigra of brains of subjects with Parkinson's disease have shown that the number of *LMX1B*-expressing neurons was reduced, correlating with the loss of dopaminergic neurons in this area (Smidt et al. *Nature Neuro.* 3:337, 2000). We hypothesize that individuals with NPS may demonstrate dysfunction in the mesencephalic dopaminergic neurons, which may present as motor abnormalities, attentional difficulties, anxiety, mood or psychotic symptoms, similar to those observed in Parkinson's disease patients.

Behavioral measures/checklists were completed by 10 female adults with NPS. These checklists have reliability and validity and are used to assess symptoms of depression, concentration, and behavior. Of the 10 subjects who completed the Beck Depression Inventory II, 5 had minimal depression symptoms and 5 had symptoms consistent with mild depression. On the NEO Personality Inventory-R, 5 of the 10 subjects reported symptoms consistent with depression, 3 with a high degree of depression. On the Conners CAARS-S:L Scale, 8 subjects completed the form and 1 had symptoms consistent with attention deficit hyperactivity disorder. On the State-Trait Anxiety Inventory, 5 of 8 subjects had trait anxiety symptoms greater than the 70th centile. Future studies will be designed with neurological testing, neuropsychological testing and psychiatric evaluations to better assess cognitive abilities, mood symptoms, motor deficits, and behavioral traits and abnormalities, and these results will be compared to the results from subjects with Parkinson's disease.

**Cranial computed tomographic findings of 20 patients with Williams-Beuren syndrome.** *S.M.M. Sugayama<sup>1</sup>, M. Valente<sup>2</sup>, J.A. Paz<sup>3</sup>, C. Leite<sup>2</sup>, L.M.J. Albano<sup>1</sup>, C.A. Kim<sup>1</sup>.* 1) Genetics, Dept Pediatrics, Instituto da Crianca, Sao Paulo, Sao Paulo, Brazil; 2) Radiology; 3) Neurology.

Williams-Beuren syndrome (WBS) is a rare neurodevelopmental disorder affecting multiple systems. The main features are typical facies, cardiovascular anomalies, mental retardation, friendly and outgoing personality and transient hypercalcemia. The syndrome is caused by a submicroscopic deletion in the chromosome region 7q11.23 which is detectable by FISH analysis in 90-95% of the patients. Reported central nervous system anomalies are decreased brain volume, overgrowth of the neocerebellum, normal development of the frontal cortical and limbic structures, underdevelopment of the cerebrum and subcortical structures and posterior temporal lobe, and Chiari I malformation. We report on CT scan findings of 20 patients (11M-9F); all sporadic cases. The main age at the diagnosis of WBS was 5.1 years. All patients presented typical elfin facies, developmental delay/mental retardation, friendly personality, skeletal and dental anomalies. Deletion of the elastin gene analysed by FISH was found in 17 patients (85%). CT scan was performed in all patients; MRI was done in 2 patients. The age at CT scan ranged between 11mo and 18y5mo (mean 8.9y). CT scan was normal in all FISH negative patients, but this exam was abnormal in 7/17 (41%) of FISH positive ones. The following anomalies were identified: agenesis of corpus callosum (1), arachnoid cyst (1), calcification of choroid plexus (3), calcification of the frontal horn of lateral ventricle (1), enlargement of adenohypophysis (1), bilateral internal capsula showing comparative hipodensity. MRI of one 4 year-old boy demonstrated mild cerebellar tonsils invagination without compressive deformity of the IV ventricle or Chiari I malformation (CMI). The majority of our patients presented nonspecific changes on CT scan/MRI. The incidence of CMI in WBS patients is unknown. Our patient is likely the tenth case of WBS patient with CMI. Shortened cranial base and underdeveloped posterior fossa may predispose WBS patients to CMI. We recommend CT scan/MRI in WBS patients because of the risk for developing fossa anomalies without neurologic symptoms.

**Structural brain abnormalities in a genetic subtype of schizophrenia.** *L.E. Scutt<sup>1</sup>, R.B. Zipursky<sup>1,2</sup>, D.J. Mikulis<sup>3</sup>, E. Chow<sup>1,2</sup>, A.S. Bassett<sup>1,2</sup>.* 1) Queen St. Site, CAMH, Toronto, ON, Canada; 2) Dept. of Psychiatry, Univ. of Toronto, ON, Canada; 3) Toronto Western Hospital, Toronto, ON, Canada.

Background: 22q11 Deletion Syndrome (22qDS) is a genetic syndrome associated with a chromosome 22q11 deletion and an increased prevalence of psychosis, and may be a genetic subtype of SZ (22qDS-SZ). Although abnormal neurodevelopment is implicated in both 22qDS and SZ, there are few studies addressing the relationship between psychosis and brain structure in adults with 22qDS. Objective: To determine if structural brain abnormalities commonly reported in SZ (enlarged ventricles and decreased temporal gray matter (GM) volumes) are associated with the expression of SZ in adults with 22qDS. Methods: Three groups were examined: 22qDS-SZ (n=12), 22qDS-NP (no psychosis, n=7) and healthy controls age and sex-matched to 22qDS-NP subjects (n=7). All subjects were quantitatively assessed with MRI scans. The three groups were compared on total and regional volumes of cerebrospinal fluid (CSF), GM and white matter (WM) using standard methods corrected for total brain volume. Group differences were assessed using ANOVA and post-hoc t-tests. Results: There were no significant group differences for sex (p=0.53) or age at scan (p=0.44). A significant effect of group was found for volumes of ventricular CSF (F=11.8, p=0.0003), sulcal CSF (F=12.7, p=0.0002) and temporal lobe GM (F=5.2, p=0.01). Post-hoc analyses revealed that the 22qDS-SZ group had greater ventricular (p=0.004), and sulcal (p=0.001) CSF and smaller temporal lobe GM (p=0.04) volumes than the 22qDS-NP group. 22qDS-NP subjects had larger ventricular CSF volumes than Controls (p=0.04); no other significant effects of group were observed. Conclusion: These results suggest that increased sulcal CSF and decreased temporal lobe GM volumes may be markers of abnormal neural development associated with the expression of SZ in adults with 22qDS, while ventricular enlargement may be a more universal finding in 22qDS.

**Detection of Y-sequences by PCR in patients with Turner stigmata.** *M. Floria, R.G.P. Queiroz, L.C. Caetano, L.H.G. Teixeira, L. Martelli, E.S. Ramos.* Department of Genetics, School of Medicine of Ribeiro Preto-SP, Brazil.

Gonadal neoplasms are frequently associated with intersex cases. Patients, who have dysgenetic gonads and a Y chromosome (normal or abnormal), have a 25-30% risk of developing gonadoblastoma. Many authors hypothesized that the gene(s) encoded by a gonadoblastoma locus on the Y chromosome (GBY) has a normal function in the testis and acts as an oncogene in the dysgenetic gonads. In some cases, structural aberrations of the Y chromosome may difficult its characterization by cytogenetics analysis. We studied 140 patients with Ullrich-Turner syndrome (UTS), by cytogenetics techniques, and selected 14 (10%) that presented a structural abnormal sex chromosome in order to determine the X or Y origin by PCR in peripheral blood samples. Among the genes so far isolated from the human Y chromosome residing on the GBY locus, we chose AMELY and TSPY. The SRY and DAZ sequences were also utilized to detect the presence of more distal regions on Yp and Yq, respectively. Molecular analysis confirmed a Y-chromosomal origin of the structural abnormal sex chromosome in two patients, but one of these showed only Yp sequences. Gonadoblastoma was confirmed in one case. The identification of the abnormal Y chromosome is important for the clinical management and prognostic counseling of the patients with UTS stigmata. Supported by: CAPES, FAEPA, CNPq, FAPESP.

**Animal model for NF1: Human neurofibroma xenotransplants into the ear of SCID mice.** *D. Babovic-Vuksanovic, B.E. Knudsen, T.B. Plummer, J.E. Parisi, L. Petrovic, S. Babovic, J.L. Platt.* Department of Medical Genetics, Department of Surgery and Transplant Biology Unit, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Neurofibromatosis (NF) type 1 is a common tumor-predisposing disease in humans, causing significant morbidity and mortality. Surgical treatment remains the only therapeutic option and it can be applied only in patients with resectable tumors. Study of other potential therapies is limited by the lack of the suitable animal model. We explored possibilities to transplant human tumors into the severe combined immunodeficiency (SCID) mice. We transplanted 21 human schwannomas and 106 human neurofibromas into various body locations in 37 experimental animals. A total of 127 tumors were implanted, 22 in the sciatic nerve and 105 in three subcutaneous locations, under the skin of the neck, back and skin of the ear. Tumor implants were monitored for survival, growth and morphological changes over a period of up to twelve months. The tumors were evaluated by direct measurement and inspection on autopsy examination, as well as by histology and immunostaining for presence of connective tissue and myelin. The survival of subcutaneously implanted tumors was slightly lower than that of tumors implanted into an epineurium of the sciatic nerve ( $p > 0.05$ ). Implants were stable in size and histologic appearance for an extended period of time. Our results indicate that human schwannomas and neurofibromas may be successfully grown in SCID mice in various subcutaneous locations. Tumor grafts implanted into the ear can be easily visualized and monitored for survival, change in size and vascularization. Therefore, human neural tumor xenotransplants into the ear of the SCID mice may be a useful experimental model for evaluation of pathogenesis and therapy.

**A new syndrome: Endodermal sinus tumor, complex CHD, polycystic-hypoplastic left kidney, dysplastic teeth, tapered fingers, and mild developmental delay.** *H. Druker<sup>1</sup>, M. Greenberg<sup>1</sup>, R. Weksberg<sup>1,2</sup>*. 1) Div of Haematology & Oncology, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, Canada.

Our patient presented initially with a polycystic-hypoplastic left kidney detected antenatally on routine ultrasound. At three months of age, she was found to have cardiac abnormalities: VSD, PDA, and pulmonary stenosis. She presented in the emergency department at 10 months of age with a three-week history of irritability, decreased oral intake and failure to thrive. At that time it was noted that she had abdominal distention and a palpable mass in the RLQ. A laparotomy was performed and a malrotation and volvulus of the bowel were corrected at which time an endodermal sinus tumor was discovered in her pelvis. On genetic evaluation at 12 years of age, she was >97centile for height, weight, and head circumference. She was noted to have downslanting palpebral fissures, slightly posteriorly rotated ears, small upturned nose, synophrys, conically-shaped teeth, tapered fingers, 2-3 foot syndactyly, and mild developmental delay. Fragile X and oligosaccharides were normal. Chromosomes and 22q- are pending. This unique constellation of findings represents a previously undescribed syndrome.

**POLYSYNDACTYLY, COMPLEXE CARDIOPATHY AND HEPATIC DUCTAL PLATE ANOMALIES : AN AUTOSOMAL RECESSIVE SYNDROME DIAGNOSED PRENATALLY.** *B. Gasser<sup>1</sup>, J. Messer<sup>2</sup>, B. Langer<sup>3</sup>, C. Stoll<sup>1</sup>.* 1) Service de Gntique Mdicale, Hpital de Hautepierre, Strasbourg, France; 2) Service de Pdiatrie 2, Hpital de Hautepierre, Strasbourg, France; 3) Service de Gynecologie 2, Hpital de Hautepierre, Strasbourg, France.

A distinct syndrome was ascertained in a 3 year-old girl and her brother. The proband was the first child of first cousin parents. She was born after an uneventful pregnancy. At birth multiple congenital anomalies were noted : ptosis of the left eyelid, hypertelorism, anteverted nares, large fontanel, long philtrum, ungueal hypoplasia, polysyndactyly, simian crease and complex cardiopathy : atrial septal defect with abnormal pulmonary venous return. Abdominal ultrasonographic examination showed hepatic cysts : a large median cyst, 2 cysts of the right lobe and many smaller cysts of the left lobe. She was succesfully operated on for her cardiopathy at 1 year of age. A normal sisters was born 2 years after the proband. During the next pregnancy of the mother, fetal ultrasonographic examination showed an hypertrophy of the right ventricle and atria, a dextroposition of the aorta, a bilateral pyelic dilatation and a club foot. The couple asked for termination of the pregnancy. Necropsy showed a facial dysmorphia, a small penis, a polysyndactyly, a ventricular septum defect and a malformation of the ductal plate. In a 17 year-old boy and his deceased sister Martinez Y. Martinez et al. (1981) reported a cardiac dysplasia, a peculiar facies, bilateral mesoaxial hexadactyly, synmetacarp, short stature, ocular torticollis, and delayed puberty. Bonneau et al. (1983) described a family in which 3 sibs had a complex cardiac malformation, hexadactyly of the first toe, and syndactyly of the third and fourth fingers. Rejab (1997) described two sibs with similar features in an Omani family. In conclusion another family has been described with features similar to the three previously reported families with complex congenital cardiopathy and polysyndactyly. However the sibs described in this report had anomalies of the ductal plate which were not reported in the other families. These new findings are in favour of autosomal inheritance of this condition which is amenable to prenatal diagnosis.

**Familial segregation of capillary malformations as an autosomal dominant trait.** I. Eerola<sup>1</sup>, L. Boon<sup>1</sup>, S. Watanabe<sup>2</sup>, A. Domp Martin<sup>3</sup>, J.B. Mulliken<sup>4</sup>, M. Vikkula<sup>1</sup>. 1) Lab. of Human Molec. Genetics, Christian de Duve Institute, Brussels, Belgium; 2) Showa Univ. School of Medicine, Tokyo, Japan; 3) CHU de Caen, Caen, France; 4) Childrens Hospital, Harvard Medical School, Boston, MA, USA.

Vascular anomalies comprise a heterogenous group of disorders the severity of which varies from life threatening diseases to cosmetic harm. They are defects of vasculogenesis/angiogenesis and thereby provide a tool to study the mechanisms involved in these processes. The first evidence of a mutation causing human vascular malformation was the linkage of a dominantly inherited venous malformation to a mutation in the *TIE-2* gene.

Capillary malformation (CM, or port-wine stain) is the most common vascular malformation occurring in 0,3% of newborns. CMs are small flat cutaneous lesions, which consist of an increased number of ectatic capillary-like channels within papillary dermis. Vascular birthmarks, such as salmon patch, are milder variants of CM that occur up to 10% of newborns. Unlike the vascular birthmarks, the reddish coloration of CMs does not disappear, but becomes darker with advancing age. CMs may cause distress when affecting large areas of e.g. facial skin. CMs are usually sporadic, yet some families have been reported.

The factors triggering the formation of CMs are not known. Similarly, it remains unknown whether the pathogenesis includes local overgrowth of capillaries or disturbance in the remodelling of the capillary plexus. Recent studies suggested that hemangiomas, another type of vascular anomaly affecting capillaries, arise from an intrinsic defect, e.g. somatic mutation, in vascular endothelial cells. In addition, evidence for genetic predisposition has been presented. In our study of CMs, we have found 26 families with autosomal dominant inheritance with incomplete penetrance. This suggests that single gene defects are involved in the genesis of CMs. Genome-wide linkage analysis on the CM families is currently under way to discover the locus/loci involved. (vikkula@bchm.ucl.ac.be).



**Glomuvenous malformation is clinically and genetically different from common venous malformation.** *L.M.*

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Venous malformations (VMs) are localized defects of vascular morphogenesis. They are single or multiple bluish-purple lesions that occur mainly in skin and mucosa (Vikkula et al., 1998). Histologically, two distinct subtypes have been identified; common VM and venous malformation with "glomus cells" (GVM). GVMs have previously been called as "multiple glomus tumors" and "glomangioma". To harmonize the nomenclature, and to stress the morphological and clinical similarity between these lesions and common VMs, we suggest to use the term glomuvenous malformation (GVM). With our genetic studies on venous anomalies (Vikkula et al, Cell 1996, and see abstracts Vikkula et al., and Brouillard et al. at this meeting), we have identified a causative gene for both subtypes, TIE2 for VMs and glomulin for GVMs. These genetic analyses gave the opportunity to clinically analyze 578 patients with VMs and 151 with GVMs for clinical characteristics, signs, symptoms, location, size, number, age at onset, and inheritance. Differences were noted for shape, colour and structure of the lesions, painfulness, and distribution. In addition, although both lesions can be inherited as an autosomal dominant trait with high penetrance, GVMs are familial in at least 67% of cases in contrast to only about 1% of VMs. Thus, these data create a basis for clinical differential diagnosis, genetic counseling and differential therapeutic approach for venous anomalies. (boon@chir.ucl.ac.be).

**Dilated cardiomyopathy in two infants with Prader Willi syndrome and cytogenetically visible microdeletion of 15q11-q14.** MU. Ahmad<sup>1</sup>, Y.S. Choy<sup>2</sup>, L.C. Hung<sup>2</sup>, S.K. Tan<sup>2</sup>, T. Malinee<sup>3</sup>, SK. Tan<sup>1</sup>, P.K.C. Lim<sup>1</sup>, A. Othman<sup>2</sup>, S.K. Ten<sup>4</sup>, T.B. Khoo<sup>2</sup>. 1) Molecular pathology, Institute of Medical Research, Kuala Lumpur, Malaysia; 2) Kuala Lumpur Hospital, Malaysia; 3) Selayang Hospital, Kuala Lumpur, Malaysia; 4) Cytogenetics, Institute of Medical Research, Malaysia.

Approximately 75% of patients with Prader Willi syndrome (PWS) have a microdeletion of paternal chromosome 15 between bands 15q11 and 15q13. Cardiac involvement in the form of cor pulmonale and pulmonary hypertension may occur in older patients with obesity and obstructive sleep apnoea. Here we report two unrelated infants with PWS presented with dilated cardiomyopathy associated with floppiness, respiratory distress and feeding difficulties. The first patient, the second child of a young and non-consanguineous couple, presented to us at 9 months of age with severe failure to thrive, psychomotor delay, mild dysmorphic facies and cardiac failure. Echocardiography revealed dilated cardiomyopathy. The second patient suffered from severe hypoxic ischaemic encephalopathy after recurrent episodes of cardiorespiratory respiratory failure due to dilated cardiomyopathy. Both infective screens and metabolic testings in these two patients were negative. Thyroid function tests were also normal. Methylation PCR confirmed the diagnosis of PWS. Fluorescence in situ hybridization using SNPRN probe (VYSIS) confirmed microdeletion of the PWS critical region. High resolution cytogenetic analysis revealed visible deletion of chromosome 15q which appeared to be between band q11-q14, a deletion larger than the PWS critical region of 15q11-q13. The deletion had probably also disrupted the cardiac alpha actin gene (ACTC) which causes one form of autosomal dominant dilated cardiomyopathy, located at 15q14.

**Paraesophageal hernia in Marfan syndrome.** *S.J. Davies<sup>1</sup>, A. Wardhaugh<sup>2</sup>, M. Nicol<sup>1</sup>, V. Booker<sup>2</sup>, D.G. Wilson<sup>2</sup>.* 1) Inst Medical Genetics, Univ Hosp of Wales, Cardiff, Wales; 2) Congenital Heart Disease Centre, Univ Hosp of Wales, Cardiff, Wales.

Hernias including inguinal, femoral and diaphragmatic are described as features of the autosomal dominant disorder Marfan syndrome (MFS). Parida et al in 1997 described a neonate with severe clinical features suggestive of neonatal MFS who had a large paraesophageal hernia. Within a 12-month period in the Welsh Paediatric Marfan clinic we have confirmed a diagnosis of MFS in 3 children who had surgery for paraesophageal hernia within the first year of life. Case 1 was diagnosed as MFS aged 14 years following acute aortic dissection in her mother. She was a term baby with a birth weight of 2.61kg. Prenatal ultrasound had suggested bilateral hydronephrosis and queried a heart anomaly. Postnatal investigation indicated a left diaphragmatic hernia. Surgery revealed a large paraesophageal hernia extending to the left. Case 2 is the brother of case 1 born at term with a birthweight of 2.8kg. He failed to thrive and had severe vomiting. At 4 months a right-sided diaphragmatic hernia was diagnosed. At operation aged 5 months a large paraesophageal hernia was confirmed with absence of the right crus of the diaphragm. He had recurrent pneumothoraces requiring pleurodesis at the time of diagnosis, aged 13. Case 3 was born at term weighing 3.4kg. She was a miserable baby who failed to thrive with vomiting and recurrent chest infections. At 6 months a chest Xray confirmed a left-sided diaphragmatic hernia. Surgical repair at 9 months showed a large paraesophageal hernia. Diagnosis of MFS was suggested at 8 years on the basis of skeletal features. Paraesophageal hernias are unusual in infancy and indicate investigation for Marfan syndrome or other connective tissue disorder. Parida SK, KrissVM, Hall BD. Hiatus /paraesophageal hernia in Neonatal Marfan syndrome. *Am J Med Genet*, 1997; 72:156-158.

**Three sibs with congenital left heart defects.** *G.C. Gowans<sup>1</sup>, P.R. Winter<sup>1</sup>, K.M. Christensen<sup>1</sup>, F.F. Yen<sup>1</sup>, B. Angle<sup>1</sup>, A. Parsian<sup>2</sup>, W.L. Sobczyk<sup>3</sup>, J.H. Hersh<sup>1</sup>.* 1) Genetics Unit, Department of Pediatrics, Weisskopf Center for the Evaluation of Children, University of Louisville, Louisville, KY; 2) Birth Defect Center, University of Louisville Health Sciences Center, Louisville, KY; 3) Division of Pediatric Cardiology, Department of Pediatrics, University of Louisville, Louisville, KY.

In the pathogenetic classification of congenital cardiovascular malformations (Clark 1996), abnormal intracardiac blood flow is thought to be the immediate etiology of Group II malformations that include perimembranous ventricular septal defect, right heart defects, and left heart defects. The left heart defects include bicuspid aortic valve, aortic valve stenosis, coarctation of the aorta, interrupted aortic arch (type A), hypoplastic left heart and aortic/mitral atresia. There has been scant evidence of genetic loci involved. We report on a 6-year-old girl, her 3-year-old brother and their 10-month-old sister with congenital left heart disease. All have bicuspid aortic valves, the two youngest have severe coarctation of the aorta, the eldest has a subvalvular membrane, and the youngest has a VSD. The two youngest also have spherocytosis, as does their mother. These children represent all the pregnancies of their parents. There is no consanguinity between the parents and there is no known congenital heart disease in either parent's families. Echocardiograms on the parents are pending. As expected, a microdeletion at band 22q11.2 was not identified using a Vysis DiGeorge/VCFS FISH probe on a blood sample from the youngest child. Pedigree analysis of this family will be presented and we are performing a genomic scan for possible involved loci.

**A population-based study of the 22q11.2 deletion.** *L.D. Botto<sup>1</sup>, R.M. Campbell<sup>2</sup>, K. Coleman<sup>2</sup>, A. Correa<sup>1</sup>, E.M. Elixson<sup>2</sup>, J.D. Erickson<sup>1</sup>, P. Fernhoff<sup>3</sup>, K. May<sup>3</sup>, R. Merritt<sup>2</sup>, L. O'Leary<sup>1</sup>, S.A. Rasmussen<sup>1</sup>.* 1) National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, Georgia; 2) Children's Healthcare of Atlanta, Atlanta, Georgia; 3) Division of Medical Genetics, Emory University, Atlanta, Georgia.

Although several studies describe the 22q11.2 deletion, population-based data are scant. Such data are needed to evaluate the impact, distribution, and clinical presentation of the deletion in the population. Our goal was to assess the population-based prevalence of the 22q11.2 deletion, its phenotype, and its impact on the occurrence of heart defects. We evaluated data on babies born from 1994 through 1999 to mothers who resided in the 5-county metropolitan Atlanta area. We matched data from the Metropolitan Atlanta Congenital Defects Program (a population-based registry with active case-ascertainment), the Sibley Heart Center at Children's Healthcare of Atlanta, and the Division of Medical Genetics at Emory University. We identified 42 children with the deletion among 255,000 liveborn infants, for an overall prevalence of 1 in 6,087 births (95 percent confidence interval, 1 in 4,505 - 1 in 8,446 births). Of the affected children, 37 (88 percent) had a heart defect, ranging from severe (truncus arteriosus) to mild (asymptomatic muscular VSD). The remainder had other congenital anomalies. Most children had dysmorphic features described in the registry records. The deletion contributed to 1 of every 65 cases of major heart defects (37/2,396). Specifically, the deletion contributed to 1 of every 2 cases of interrupted aortic arch, 1 of every 4 cases of truncus arteriosus, and 1 of every 9 cases of tetralogy of Fallot. In this population-based study, the 22q11.2 deletion was common, though the prevalence we found likely represents a minimum estimate. Assuming the same prevalence nationwide, one can estimate that over 600 similarly affected babies are born yearly in the United States. Population-based estimates such as these will be useful to health care planners and providers to estimate the resources needed for the optimal acute and long-term care of affected individuals.

**Hereditary Haemorrhagic Telangiectasia -delineation of the different phenotypes caused by endoglin and ALK1 mutation.** *J. Berg*<sup>1</sup>, *M. Porteous*<sup>2</sup>, *D. Reinhardt*<sup>2</sup>, *S. Holloway*<sup>2</sup>, *C. Gallione*<sup>3</sup>, *A. Lux*<sup>4</sup>, *W. McKinnon*<sup>5</sup>, *A. Guttmacher*<sup>6</sup>, *D. Marchuk*<sup>3</sup>. 1) Division of Medical and Molecular Genetics, GKT School of Medicine, London, UK; 2) South-East Scotland Regional Genetics Service, Western General Hospital, Edinburgh, UK; 3) Department of Genetics, Duke University Medical School, North Carolina, USA; 4) Inst. f. Molekularbiologie u. Zellkulturtechnik, Mannheim, Germany; 5) Vermont Regional Genetics Center, Burlington, USA; 6) NHGRI, Bethesda, MD.

Hereditary Haemorrhagic Telangiectasia (HHT) is an autosomal dominant vascular dysplasia characterised by mucocutaneous telangiectasis, epistaxis, gastrointestinal haemorrhage and arteriovenous malformations in the lung and brain. Causative mutations for HHT have been identified in two different genes, endoglin and ALK1. From a postal questionnaire survey, we have obtained clinical data from 49 patients with a known endoglin mutation and 34 patients with a known ALK1 mutation. Plotting Kaplan-Meier survival curves and using the log rank test to compare the two groups, we have shown that patients with endoglin mutation have an earlier onset of epistaxis ( $P=0.01$ ) and telangiectasis ( $P=0.0001$ ). Brain arteriovenous malformations were present in 4/49 patients with endoglin mutations and 1/34 patients with ALK1 mutations (not significant). Pulmonary arteriovenous malformations were reported in 17/49 patients with endoglin mutations but 0/34 patients with ALK1 mutations ( $P<0.001$ ). We conclude that the HHT phenotype caused by endoglin mutation (HHT1) is distinct and more severe than HHT caused by ALK1 mutation (HHT2). This has significant implications for diagnosis, screening and treatment in the two different forms of HHT as well as for the understanding of the pathogenesis of the disease.

**The first autopsy series in patients with a 22q11.2 deletion.** *D.S. Huff<sup>1</sup>, D.M. McDonald-McGinn<sup>2</sup>, E.H. Zackai<sup>2</sup>.* 1) Department of Pathology; 2) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA.

Of 413 patients with a 22q11.2 deletion, 20 expired, of whom 13 had autopsies available for review. 9 were female. Age at death ranged from stillbirth to 1.9 years. All died within the first 6 months of life; 7 within the first three weeks. Heart defects were the cause of death in 8/11 with complete autopsies. 1 with interrupted aortic arch died of tracheal agenesis, 1 died due to prematurity. The cause of the stillbirth was not determined. The thymus was absent in 3 & hypoplastic/ectopic in 7. No parathyroids were found in 5; 1 in 6 & 3 in 1. Additional anomalies were variable: 1 F-anisosplesia form of heterotaxy; 2 laryngotracheal defects: 1 tracheal agenesis with esophageal fistula, 1 malformed larynx & abnormal thyroid cartilage; 4 had abnormal pulmonary lobation, 2 with azygous lobes; 2 had small congenital diaphragmatic hernias (1 left & 1 right). No major GI malformations were found, but one patient had a short attachment of the small bowel mesentery. 2 had urinary tract anomalies; 1 right renomegaly, 1 absence of the right kidney, ureter and trigone. 3 had genital tract anomalies: 1 bilateral undescended testes at the scrotal entrance with Leydig cell hyperplasia, 1 left unicornuate uterus, 1 bilateral Mullerian agenesis. The thyroid gland was abnormal in 2: 1 absent isthmus, 1 abnormally-shaped with cysts. 3 patients had anomalies of the CNS: 1 focal cerebellar dysplasia, 1 diffuse cerebral dysplasia and a right parietal A-V malformation, & 1 craniosynostosis. In summary, patients with a 22q11.2 deletion have a known pattern of anomalies, most often including cardiac defects, hypoparathyroidism and thymic aplasia/hypoplasia. We present the first autopsy series in patients with a confirmed 22q11.2 deletion and describe additional anomalies not typically associated with the deletion. Significant findings which have implications for the care of surviving patients include: laryngotracheal esophageal abnormalities, splenic hypoplasia, heterotaxy, diaphragmatic hernia, and genito-urinary findings.

**Vasomotor Instability in Chromosome 22q11 deletion.** *V. Shashi, M.N Berry, M.H Hines.* Pediatrics, Wake Forest Univ Sch Med, Winston-Salem, NC.

Approximately, 70% of individuals with chromosome 22q11 deletion have congenital heart disease, frequently requiring surgical intervention. These conotruncal heart defects are thought to be due to abnormal neural crest migration and division. A host of other vascular problems such as tortuous carotid arteries, Raynaud's phenomenon, and small veins have been reported. These, in addition to unexplained hypotension, hypertension and hypothermia raise the possibility that there may be abnormal autonomic regulation of the vascular system (personal communication, Dr. Shprintzen). Thus far, however, there has been no formal report of autonomic dysfunction in patients with 22q11 deletion. We present two neonates with 22q11 deletion who had profound hypotension. One infant had preoperative hypotension. Both had uncomplicated surgeries for congenital heart disease, but post-operatively had unexplained hypotension that was not responsive to vasopressor treatment (and extracorporeal membrane oxygenation in one infant), resulting in death, due to multi-organ system failure. Obvious causes for the hypotension, such as poor cardiac contractility, prolonged circulatory arrest and neurological abnormalities were excluded. No abnormality was found on autopsy to account for the hypotension. We hypothesize that these infants died of severe hypotension due to dysautonomia and that this is a variable feature in individuals with 22q11 deletion. The autonomic nervous system, which is responsible for blood regulation, is formed from the neural crest cells. Thus, given that the neural crest abnormalities lead to the conotruncal heart abnormalities, abnormal autonomic regulation could also be linked to the neural crest. Our hypothesis has implications for the post-surgical management of patients with 22q11 deletion. Prior knowledge of the possibility of dysautonomia would be helpful in monitoring and treatment. Further studies on this topic would help in establishing the association between 22q11 deletion and dysautonomia.



**A new autosomal dominant syndrome with hypertrophic cardiomyopathy, heterotaxy, conduction abnormalities, atrial septal defect and pulmonary stenosis.** *M. Wessels<sup>1</sup>, S. Spitaels<sup>2</sup>, R. De Krijger<sup>3</sup>, T. Cohen-Overbeek<sup>1</sup>, M. Niermeijer<sup>1</sup>, P. Heutink<sup>1</sup>, P. Willems<sup>1</sup>.* 1) Dept of Clin. Genetics, Erasmus University, Rotterdam, The Netherlands; 2) Dept of Cardiology, Univ Hosp Rotterdam, The Netherlands; 3) Dept of Pathology, Univ Hosp Rotterdam, The Netherlands.

We present a family of 9 patients with a new autosomal dominant combination of hypertrophic cardiomyopathy, conduction abnormalities, secundum atrial septal defect and situs ambiguus. A triplet was diagnosed prenatally with hypertrophic cardiomyopathy and bradycardia in all three fetuses. One female fetus had a massive hypertrophic cardiomyopathy, pulmonary atresia, tricuspid dysplasia and an absent vena cava inferior with azygous continuation. The girl died shortly after birth and autopsy additionally revealed bilateral bi-lobed lungs, polysplenia and intestinal malrotation. Her triplet brother showed valvular pulmonary stenosis, jejunum atresia, intestinal malrotation and polysplenia. The third triplet only had bradycardia and malrotation of the bowel. Examination of the father of the triplets revealed apical concentric ventricular hypertrophy, atrial septal defect, sinus bradycardia, and bilateral bi-lobed lungs. His sister was operated in childhood on a atrial septal defect and valvular pulmonary stenosis. She additionally had absent vena cava inferior, ventricular hypertrophy, sinus bradycardia, polysplenia but normal situs. A brother was known with cardiomyopathy and sick sinus syndrome. The grandfather had apical hypertrophy of both ventricles, atrial septal defect, atrial fibrillation, left bundle branch block and polysplenia. His brother and one of his 6 sisters were diagnosed with hypertrophic cardiomyopathy. Although many hypertrophic cardiomyopathies with autosomal dominant inheritance have been described, the association with structural cardiac defects and heterotaxy represents a new syndrome. A genome-wide screen resulted in a maximal lod score approximating 3 without recombination with markers on chromosome 6p23-24. This region holds the endothelin 1 gene, which might be a candidate gene as endothelin 1 knockout mice show cardiac anomalies including septal defects and outflow tract abnormalities.

**A Jagged 1 gene mutation for abdominal coarctation of the aorta in Alagille syndrome.** *A. Raas-Rothschild<sup>1</sup>, E. Shteyer<sup>2</sup>, I. Lerer<sup>1</sup>, A.J.J.T Rein<sup>3</sup>.* 1) Human Genetics, Hadassah University Hospital, Jerusalem, Israel; 2) Department of Pediatrics, Hadassah University Hospital, Jerusalem, Israel; 3) Department of Pediatric Cardiology, Hadassah University Hospital, Jerusalem, Israel.

Structural cardiac defects such as peripheral pulmonary stenosis are well described in Alagille syndrome (AGS), which is transmitted by an autosomal dominant inheritance. Haploinsufficiency of the Jagged 1 gene (JAG1) has been shown to cause Alagille syndrome. Abdominal coarctation is an uncommon vascular congenital anomaly and has been described only twice in AGS. We report on a third patient with coarctation of the abdominal aorta in AGS and right subclavian stenosis. Recently, Loomes et al (Hum Mol Genet 1999; 8(13): 2443-2449) have shown that Jagged1 gene is expressed in the developing heart and in multiple associated vascular structure including the descending aorta. Jones et al (J Med Genet 2000;37:658-662) studied the tissue expression of JAG1 in human embryo and noticed that JAG1 was also expressed in the developing aorta. We performed molecular analysis of the Jagged1 gene in this third patient with AGS and abdominal coarctation and found a mutation deletion (1485 Del CT). This corroborates with Loomes and Jones studies and confirms that coarctation of aorta might be a part of the Alagille syndrome clinical findings.

**Zinc and Immune Function in Down Syndrome.** *M.L. Soto-Alvarez<sup>1</sup>, F. Alvarez-Nava<sup>1</sup>, A.E. Rojas-Atencio<sup>1</sup>, R. Gonzalez<sup>1</sup>, J. Caizalez<sup>1</sup>, V. Granadillo<sup>2</sup>, D. Fernandez<sup>2</sup>, A. Ocando<sup>2</sup>, A. Vasquez<sup>2</sup>, J. Herrera<sup>3</sup>, W. Fulcado<sup>3</sup>.* 1) Unidad de Genetica Medica, Univ del Zulia, Maracaibo Zulia, Venezuela; 2) Laboratorio de Instrumentacion analitica-LUZ; 3) Hospital Clinico de Maracaibo.

Down syndrome (DS) is the most common cause genetics of mental retardation, with a occurrence in 1:600 - 1:800 liveborn. Their high frequency and great clinical importance makes that this syndrome is the chromosomal anomalies most studied in human. Several studies have reported alteration of the plasma level of zinc and dysfunction of the immune system in these patients which it has associated with infectious illnesses. This represents one of the main causes of mortality in DS. To determine the plasma levels of zinc and to evaluate the immune system in patients with DS. Blood samples of 40 patients (18 females with mean age 3 year, and 22 males with mean age of 4 year) with SD. Were these patients were recluted from Unit of Medical Genetics of the University of Zulia- Venezuela. Twenty samples of healthy children were used as controls. Karyotypes were carried out according to technique of Moorhead. Plasma levels zinc were determined for atomic absorption spectrophotometry and flow cytometry were used to evaluate immune systems. All patients exhibit free trisomy chromosome 21. In regarding to the plasma levels zinc and evaluation of the immunity, 10/40 patients (25%) had the values inside normal limited; 20/40 patients (50%) presented levels of zinc below the normal values. Of these, 45% had alterations in the cellular immunity and 5% in the immunity humoral, Twenty five percent (10/40) patients presented normal levels of zinc with alterations of the cellular immunity. Several investigators have reported that the zinc is an essential cofactor in the answer of cells T , phagocityc function and possibly in the production of antibodies. These results agree with other studies and suggest that the deficiency of zinc in the patients with DS affects the immune system.

**Clinical and genetic analysis of a distinct dominantly inherited spinocerebellar ataxia with male sterility and mental deficiency.** *C.M. Gomez, G.Y. Yu, D.T. Xie, J. Pryor, J.H. Anderson, S. Frutiger.* Dept Neurology, Univ Minnesota, Minneapolis, MN.

The dominantly inherited spinocerebellar ataxias (SCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by progressive imbalance, dysarthria and incoordination. Genetically distinct subtypes differ clinically, due in part to involvement of extracerebellar neuronal populations. We identified a 5-generation kindred with progressive ataxia in which all affected males are infertile. The affected individuals have an extremely broad range of onset with progressive cerebellar deficits developing sometime between childhood and the sixth decade. Detailed neuropsychological evaluations have demonstrated cognitive impairment with borderline IQ scores, especially in males. Magnetic resonance brain imaging demonstrates cerebellar atrophy. Nerve conduction studies reveal an axonal sensory neuropathy. Four of five affected males examined had testicular atrophy and six of six had azoospermia, with reduced or absent sperm. Testicular biopsy has demonstrated Sertoli-cell-only syndrome in one sterile male. Cytogenetic studies detected no chromosome anomalies or microdeletions. Mutational analyses have ruled out SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12 and fragile X. Linkage analysis has excluded SCA4, SCA5, SCA10, SCA11, SCA13, SCA14, and the entire X chromosome. Clearly, this family represents a clinically and genetically new form of SCA. A genome-wide linkage scan may locate the disease locus. Identification of the gene responsible for this unique phenotype may shed additional light into the pathogenesis of ataxia, mental retardation, and male infertility.

**Morphological characterization and genotype phenotype correlation in SHFM.** *D. Basel<sup>1</sup>, S. Sifakis<sup>1</sup>, M. Arici<sup>2</sup>, P. Beighton<sup>3</sup>, C. Delozier-Blanchet<sup>4</sup>, M.W. Kilpatrick<sup>1</sup>, P. Tsipouras<sup>1</sup>.* 1) Paediatrics, UCONN Health Centre, Farmington, CT; 2) Diagnostic Imaging and Therapeutics, UCONN Health Centre, Farmington, CT; 3) Human Genetics, University of Cape Town, South Africa; 4) Medical Genetics, University of Geneva Medical School, Switzerland.

Split Hand Foot Malformation (SHFM) is a condition, with an incidence of 1:18000, characterized by median clefts, syndactyly and bone aplasias/hypoplasias without the involvement of ectodermal structures. However, 40% of individuals presenting with this abnormality, have associated anomalies suggestive of a syndromic entity. Given the clinical and genetic heterogeneity of non-syndromic SHFM we attempted to define the nosological spectrum of the condition and investigate the possibility of phenotype/genotype correlation. We reviewed radiographs from 15 affected individuals, and in addition analyzed the phenotypes of four large families, two of which have been genetically linked to 3q27 and two to 10q24. We categorized the phenotypic manifestations of SHFM into a core of skeletal defects which were then used to correlate phenotype to genotype in this large cohort of patients. Three groups of skeletal manifestations were identified: monodactyly, bidactyly and oligodactyly, the latter frequently associated with syndactyly and a median cleft. The non-core elements include clinodactyly, polydactyly and triphalangeal thumbs. Our findings showed that the pattern of malformations in the families studied was highly variable and within the same family, affected individuals presented with a combination of the core manifestations and associated anomalies. All four extremities were not necessarily affected and symmetrical changes were uncommon. The composite phenotype of the four large families studied did not differ substantively and thus no apparent phenotype-genotype correlation was noted. Oligodactyly, bidactyly and monodactyly represent a continuum of phenotypic severity, attributable to a common developmental defect of the apical ectodermal ridge. The co-segregation of these manifestations in the same individual suggests that stochastic events influence local developmental mechanisms in determining the final phenotypic outcome.

**Comparison of the Autism Diagnostic Interview-Revised (ADI) Restrictive/Repetitive Behaviors and the Aberrant Behavior Checklist (ABC) Stereotypy Scores.** *R.K. Abramson<sup>1</sup>, M.L. Cuccaro<sup>1</sup>, K. Wieduwilt<sup>2</sup>, S.A. Ravan<sup>1</sup>, K. Decena<sup>2</sup>, H.H. Wright<sup>2</sup>.* 1) WS Hall Psychiatric Inst, Columbia, SC; 2) Univ. South Carolina School of Medicine.

Autistic Disorder (AD) is a complex neurogenetic disorder with core impairments in social, communicative, and behavioral functioning. The ADI (Lord et al, 1994) is the standard instrument for the diagnosis of AD. Refinement of phenotype to better understand homogeneous clinical subgroups may be useful in dissecting genetic etiology in AD. Cuccaro et al, 2000, conducted a factor analysis of 13 ADI items believed to represent restrictive repetitive interests and found 2 factors. Factor 1 (ADI items 72,72,77,81,84) was related to sensory repetitive behavior and Factor 2 (ADI items 73,74,75) reflected insistence on sameness. The ABC (Aman & Singh, 1986) is a standardized measure of behavior in individuals with developmental disorders, providing 5 subscores, one of which is stereotypy. This study examines whether the two scales, the ADI restrictive repetitive behaviors (ADI-RRB) and the ABC (ABC-S) stereotypy subscales are related and if ADI Factors 1 and 2 refine the relationship between the ADI-RBB and the ABC-S. Males (n=122) and females (n=44), mean age=8±4.4 yrs with AD were enrolled in the study. There were no significant gender differences on the ADI-RBB algorithm score or on the ABC-S score or on the individual items which make up the scores. The ADI-RBB algorithm score and the ABC-S were significantly correlated, Pearson Correlation = 0.282, p(2-tailed)= 0.001. For the same group, the relationship between Factor 1 (ADI-RBB) and the ABC-S was evaluated. Factor 1 (ADI-RBB) and the ABC-S were highly correlated, Pearson Correlation =0.482, p(2-tailed)=0.000. Factor 2(ADI-RBB) and the ABC-S were not correlated. Use of either Factor 1 (ADI-RBB) or the ABC-S may further refine phenotype for linkage analysis of AD.

**Trisomy 12p syndrome: A unique case with involvement of 12q.** *J.E. Martinez<sup>1</sup>, M.C. Panero<sup>2</sup>, C.M. Tuck-Muller<sup>1</sup>, W. Wertelecki<sup>1</sup>.* 1) Dept Medical Genetics, Univ South Alabama, Mobile, AL; 2) Serv. de Medicina Genética Hospital de Niños Santísima Trinidad, Córdoba, Argentina.

Trisomy 12p syndrome is a recognizable condition presenting with normal to large size, characteristic craniofacial dysmorphism and severe psychomotor retardation. Most of the 33 reported cases are the result of familial reciprocal translocations (27/33). We report a new case with a de novo 12pter-q13 duplication, a distinct cytogenetic abnormality not reported thus far. A male infant was ascertained at birth when bilateral cryptorchidism, hypospadias and post-axial polydactyly of hands were noted. An ASD with significant left to right shunt required surgical correction. He later developed seizures and abnormal EEG findings. A brain CT scan at age 4 months was unremarkable. Examination at three years of age revealed large size, macrocephaly, high and broad forehead, shallow orbits, temporal alopecia, prominent cheeks, flat nasal bridge, short and anteverted nose, long and broad philtrum, large mouth, everted lower lips, thin upper vermilion, low set dysplastic ears, bilateral simian creases and a gap between first and second toes. The child has profound mental retardation, universal hypotonia, feeding and walking difficulties, hearing loss and no speech. Cytogenetic analysis revealed a 47,XY,del(12)(q13)[6]/46,XY[22] karyotype. Parental chromosomes were normal. Attempting to correlate phenotypic manifestations and cytogenetic findings, a clinical classification in five categories based on the extent of the duplication, the involvement of other chromosomes, and the presence of mosaicism was proposed by Allen et al (1996). Only three cases have been previously observed with involvement of 12q (category V), and two of them included other abnormalities derived from parental translocations. A child with a de novo mosaic trisomy of 12pter-q11 had striking similarities with our patient except for the absence of a heart defect. However, only subtle clinical differences were noted between our case and patients in other proposed categories, suggesting that involvement of proximal 12q and mosaicism, do not significantly change the phenotypic expression of this syndrome.

**Adjunct diagnostic test for Angelman syndrome: The tuning fork response.** *B.D. Hall.* Dept Pediatrics/Genetics, Univ Kentucky Col Med, Lexington, KY.

I report an apparently unique response by Angelman syndrome individuals to the vibrating tuning fork when it is held up to their ear. The response is a wide smile with an almost simultaneous outburst of laughter. A total of 5 consecutive individuals (only 1 of whom was confirmed at the time to have Angelman syndrome) with ages ranging from 3 4/12 years to 38 years, displayed this unique response. No such response was obtained from normal children/adults or individuals with fragile X, microcephaly, autism, or other multiple congenital anomaly syndromes.

The response to the vibrating tuning fork in normal individuals or those with other syndromes is quite different. They will either look forward, with a cautiously curious expression, or immediately pull away with an anxious appearance, with or without crying or screaming. Some individuals do adjust to the sound of the tuning fork and may later smile or occasionally laugh. Consequently, repetition may give a false positive response.

The tuning fork response in potential Angelman syndrome patients is a quick test that may strengthen or reduce your suspicion of Angelman syndrome. I do not know if it is useful in children under 3 years of age, but I am presently testing a 17-month-old girl.

Photographs of individual responses to the tuning fork will be presented.



**A novel autosomal dominant cerebellar ataxia associated with tremor. - Clinical features and molecular genetic analysis.** *K. Hara*<sup>1</sup>, *T. Shimohata*<sup>1</sup>, *M. Oyake*<sup>1</sup>, *H. Ishiguro*<sup>2</sup>, *K. Hirota*<sup>2</sup>, *T. Matsuura*<sup>3,4</sup>, *T. Ashizawa*<sup>3,4</sup>, *S. Tsuji*<sup>1</sup>. 1) Neurology, Brain Res Inst. Niigata univ, Niigata, Japan; 2) Department of Neurology, Akita Red Cross Hospital, Akita, Japan; 3) Department of Neurology, Baylor College of Medicine, Houston, Texas, USA; 4) Neurology Service, Veterans Affairs Medical Center, Houston, Texas, USA.

Dominantly inherited, late onset pure cerebellar ataxias are genetically heterogeneous neurodegenerative disorders. Although approximately half of the families with these disorders in the Japanese population have been shown to be caused by expansion of CAG repeats in the CACNA1A gene on chromosome 19p13 (SCA6), causative genes for the rest remain to be elucidated. We have identified a three-generation family with unique clinical presentations including cerebellar ataxia and tremor. Among the 10 family members, 6 (3 males and 3 females) were affected. The mean age of onset was 37.5 years (27~47). All the affected members showed cerebellar ataxia. Furthermore, three patients had neck tremor at rest, one patient had hand tremor at rest, and one patient had both. The age at onset of the patient exhibiting hand and neck tremor in addition to cerebellar ataxia was 27 years, which is earlier than that of her affected father (47 years) and, furthermore, earlier than those of the other affected individuals, suggesting the possibility of anticipation. Brain MRI showed cerebellar atrophy without brainstem atrophy. The diagnosis for SCA1, SCA2, MJD/SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, or DRPLA was excluded by mutational analysis. The possibility for SCA4, SCA5, SCA11, SCA13 or SCA14 was also excluded by linkage analysis. Interestingly, linkage to the SCA10 locus was suggested with the highest pairwised LOD scores of 2.41 at D22S928 and D22S1141(theta=0.0). Expansion of ATTCT repeat in SCA10 gene, however, was not found. Epileptic seizures, a hallmark for SCA10, were not observed in the affected patients in this family. These results suggest that this family has a novel autosomal dominant cerebellar ataxia distinct from any of previously known SCAs.

**Identification of disease causing genes in 7p11-p14 deleted in patients with Greig syndrome and additional clinical features.** *P.M. Kroisel<sup>1</sup>, K. Wagner<sup>1</sup>, D. Kolozsvari<sup>2</sup>, C. Windpassinger<sup>1</sup>, S.W. Scherer<sup>2</sup>, E. Petek<sup>1</sup>.* 1) Med. Biology & Human Genetics, University of Graz, Graz, Styria, Austria; 2) Department of Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

We report on five Greig cephalopolysyndactyly syndrome (GCPS) patients, including one pair of monozygotic twin boys with a de novo microdeletion involving the chromosomal band 7p13, where various clinical features in addition to GCPS were recognized. Besides the twin pair all patients are unrelated. Since there is a considerable lack of well defined clinical characterizations of the few patients with microdeletions involving 7p13 with GCPS described so far, here we want to focus on the delineation of the phenotype in all patients in particular on those symptoms, that are not typically related to GCPS like a moderate psychomotor retardation, seizures, muscle fibre anomalies, cardiac anomalies, hyperglycaemia or hirsutism. In order to more easily characterize the size of the different microdeletions, we obtained, starting from lymphoblastoid cells of the patients, monoallelic hybrid cell lines. By screening with region specific STRs all deletion breakpoints were precisely mapped and based on these results in combination with genomic sequence data available by now, we were able to identify several candidate genes mapped to the deleted chromosomal segments.

**Parent of origin does not determine phenotype in the 22q11.2 deletion.** *D.M. McDonald-McGinn, D.A. Driscoll, M. Tonnesen, K. Sullivan, R. Kirschner, E. Goldmuntz, S. Weinzimer, D. Lynch, P. Wang, E. Moss, M. Gerdes, C. Solot, C. Catania, S. Saitta, B.S. Emanuel, E.H. Zackai.* The Children's Hospital of Philadelphia, Philadelphia, PA.

We previously reported findings on 250 patients with the 22q11.2 deletion (McDonald-McGinn et al., 1999), the majority of whom had the same large 3-4 megabase deletion. Males and females were equally likely to be affected and parent of origin studies revealed equal numbers of maternally and paternally inherited deletions. There was extreme variability within this cohort and no genotype-phenotype correlations could be made. Recently Eliez et al., 2001 reported significant differences in brain development in 18 patients with the deletion based on the parent of origin. Therefore, we undertook a study to examine this question more broadly. Fifty-nine de novo triads underwent parent of origin studies. Thirty were maternally inherited and 29 were paternally inherited. We reviewed these patients records to identify the presence of: congenital heart disease; palatal defects; immune deficiency; hypocalcemia; learning disability; language delay; articulation errors; and severity of cardiac disease. Specifically, of 41 patients with congenital heart disease, 24 were paternally inherited, of 20 with severe congenital heart defects, 11 were paternally derived; of 29 with definitive palatal abnormalities, 17 were paternal; of 11 with hypocalcemia, 6 were paternal; of 7 with structural brain abnormalities, 3 were paternal; of 15 with learning disabilities, 8 were paternal. This distribution was not statistically significant from the expected 1:1 ratio maternal versus paternal origin based on Chi-square analysis. The immune system including CD3, CD4, CD8, CD19 and NK cells and language delays and articulation errors were not statistically different based on SD calculations.

**Familial Pulmonary Fibrosis in the USA.** *M.M. Wahidi<sup>1</sup>, M.C. Speer<sup>1</sup>, M. Steele<sup>2</sup>, K.K. Brown<sup>2</sup>, M.I. Schwarz<sup>2</sup>, D.A. Schwartz<sup>1</sup>.* 1) Medicine, Duke University Medical Center, Durham, NC; 2) National Jewish Coalition, Denver, CO.

Pulmonary fibrosis is a rare form of lung disease that affects the interstitium of the lungs and often fatal within 3-5 of diagnosis. Families with at least two members affected by PF are recruited through a network of collaborators and advertisement on the Internet ([www.fpf.duke.edu](http://www.fpf.duke.edu)). Data on affected members includes a standardized clinical history and risk factor questionnaire, pedigree, DLCO, CXR, HRCT, and lung biopsy (when available). Asymptomatic relatives are screened with a questionnaire, DLCO, and CXR. Thirty families including 108 affected individuals have been identified. Males were more frequently affected than females (55%). The predominant race was Caucasian (94% Caucasian, 6% Hispanic). Smoking was identified in 42% of cases with available data. Exposure to known fibrotic agents such as asbestos, silica, or wood dust was recorded in 19% of cases. Mean age at diagnosis was 59 (sd=15.8) and mean age of death was 62 (sd=15.5). The diagnosis of PF was confirmed by an open lung biopsy and autopsy in 24 patients (22%). A high resolution CT scan of the chest was obtained in 47 patients (43%). In the 24 families with at least 2 documented cases, PF occurred in multiple family members in 15 families. Ten families have at least one affected sibling pair, 4 have affected parent-child pairs including one pair of affected father and son, and 4 pedigrees include more distantly related affected relative pairs. The familial aggregation documented in these families is consistent with a genetic basis in at least a subset of PF cases.

**Clinical and Genetic Aspects of Trigonocephaly: A Study of 22 Cases.** *C. Azimi*<sup>5</sup>, *S.J. Kennedy*<sup>1,3,4</sup>, *C. Forrest*<sup>2,3,4</sup>, *A.S. Teebi*<sup>1,3,4</sup>. 1) Div. of Clinical & Metabolic Genetics; 2) Div. of Plastic Surgery; 3) Hospital for Sick Children; 4) Toronto, ON, Canada; 5) Tehran Univ. of Med. Sciences, Tehran, Iran.

We reviewed 22 patients ascertained to have trigonocephaly/metopic synostosis as a prominent manifestation. In 15 patients, trigonocephaly was the only significant finding (68%) while in 7 patients trigonocephaly was a part of a syndrome (32%). Among the non-syndromic cases, 10 were males and 5 were females with a sex ratio of 2M:1F. Only 1 patient had a clearly affected parent (6.6%). All patients had normal psychomotor development. Among the syndromic cases, 3 had Jacobsen syndrome, due to deletion of chromosome 11q23 (43%). Of the remaining 4 syndromic cases, one patient had Say-Meyer syndrome, a possible X-linked recessive disorder. Another patient had multiple congenital anomalies and bilateral retinoblastoma. This patient did not have a detectable deletion in chromosome 13q14.2 by chromosomal analysis and FISH. One patient had additional sagittal synostosis, peculiar facies, Morgagni diaphragmatic hernia, stippled epiphyses, and mild psychomotor delays. A peroxisomal disorder was suspected but never confirmed. The final patient had severe micrognathia, large ears, atrioventricular septal defect, symmetrical cutaneous syndactyly of hands and feet, multiple café-au-lait spots and developmental delay in addition to trigonmicrocephaly. These findings were suggestive of a new MCA/MR syndrome. This patient was investigated for a cryptic chromosomal rearrangement, utilizing FISH and SKY, and was found to have a normal karyotype. In two of the syndromic cases FGFR2 and FGFR3 genes were studied, but no mutations were detected.

This study demonstrates that the majority of cases of non-syndromic trigonocephaly are sporadic and benign, apart from cosmetic effects on appearance. Syndromic cases involving trigonocephaly are heterogeneous with regard to their etiology and include both chromosomal and single gene disorders. This study emphasizes the importance of investigating patients with non-syndromic trigonocephaly for chromosome abnormalities.

**Goeminne Syndrome - Expansion of a rare X-linked phenotype.** *B.A. Fernandez<sup>1</sup>, A.E. MacMillan<sup>1</sup>, M. Crowley<sup>1</sup>, D. Jewer<sup>2</sup>, S.W. Scherer<sup>3</sup>.* 1) Faculty of Medicine, Discipline of Genetics, Memorial University of Newfoundland, St John's, NF; 2) Faculty of Medicine, Dept.of Surgery, Div. of Plastic Surgery, Memorial University, St John's, NF,; 3) Dept. of Genetics, The Hospital for Sick Children, 555 University Ave, Toronto, ON, M5G 1X8.

In 1968, Goeminne described a syndrome of congenital progressive torticollis, multiple keloids, and cryptorchidism in an Italian family with 4 affected males and 2 affected females. The males were more severely affected than females and McKusick (OMIM # 314300) has characterized Goeminne syndrome as X-linked with incomplete dominance. The only other reported cases are 2 females with partial features of the syndrome, each with an apparently balanced X;autosomal translocation. These female patients have been used to map a locus to Xq28, distal to *G6PD*.

We report a second male proband with this rare syndrome. He is 44 years old and was born with right-sided muscular torticollis, scoliosis and bilateral cryptorchidism. He is infertile and of normal intelligence. In mid-childhood, he developed spontaneous keloids, as well as disfiguring keloids at surgical incision sites. The first keloid formed at 5 years at the site of sternocleidomastoid surgery. Spontaneous keloids developed on the soles of the feet at 6 years. At 15, Harrington rods were removed and a thick linear keloid formed along the spinal incision.

The proband has a number of anomalies not described in Goeminne's pedigree. In the 3rd decade, he developed bilateral Dupuytren contractures, and keloids formed at the release sites. Additionally, he has macrocephaly, broad thumbs and great toes, and dysmorphic facial features including: prominent supraorbital ridges; telecanthus; a retruded midface; a wide mouth and large low set ears. His 75 year old mother may also be affected. She has Dupuytren contractures and with less aggressive keloid formation. The proband's routine karyotype was normal, as was *FISH* for the 16p13.3 (Rubinstein-Taybi) locus. Molecular studies of the Xq28 region using a candidate gene approach are in progress.

**Further Characterization of Odontotrichomelic Syndrome.** *M.N. Berry, V. Shashi.* Dept. Pediatrics, Division of Medical Genetics, Wake Forest Univ. School of Medicine, Winston-Salem, NC.

Odontotrichomelic syndrome (OS) is a rare, autosomal recessive condition associated with tetramelic deficiencies, ectodermal dysplasia and facial differences. We report on the features of a 31-year-old female who has upper limb reduction defects, abnormal facial features and findings consistent with ectodermal dysplasia. Although scant information is known about the maternal and paternal family histories, the patient was not aware of a family history of limb defects or ectodermal dysplasia. She reports that she has unilateral hearing loss, velopharyngeal insufficiency, an esophageal web, a resolved heart murmur, decreased sweating and hypodontia. She underwent surgical repair for protuberant ears. Her IQ is normal. On exam, her height, weight and head circumference are <<5th centile. She has sparse eyebrows, lowset ears, extremely small lower lacrimal punctae, absent upper lacrimal punctae, a large nose, prominent lips, a high arched palate, soft, slightly dry and prematurely wrinkled skin, scant body hair and thoracolumbar scoliosis. The right forearm is shortened with a missing radius. There is a radial club hand on the right with 3 well-formed digits, a bifid digit in place of the thumb and a cleft between the thumb and other digits. Her left upper limb is shortened, the forearm is missing, and there is ectrodactyly of the hand. There are only 2 digits on the left hand. Except for syndactyly of the 3rd and 4th left toes, the lower limbs are normal. We feel the features of limb reduction defects and ectodermal dysplasia in our patient suggest OS. We considered other ectodermal dysplasia conditions such as Ectrodactyly-Ectodermal dysplasia-Cleft lip/palate syndrome in the differential diagnosis. Although our patient has ectrodactyly and ectodermal dysplasia, the severity of the upper limb reduction defects and the typical facial features seen are more compatible with OS. Thus, our report expands the phenotype of OS and is the 4th fully described case of this rare condition.

**Functional renal abnormalities in two children with Kabuki syndrome.** *D. Genevieve*<sup>1</sup>, *C. Loirat*<sup>2</sup>, *C. Baumann*<sup>1</sup>. 1) Service de genetique, Hopital Robert Debre, Paris, France; 2) Service de Nephrologie Pediatrique Hopital Robert-Debre, Paris, France.

Kabuki (Niikawa-Kuroki) syndrome (KS) is a rare MCA/MR characterized by post natal growth retardation, mild mental retardation, a characteristic facial dysmorphism, skeletal malformations and dermatoglyphic abnormalities. Visceral malformations including renal malformations are frequent in this syndrome. Here we report, for the first time, on two patients with functional renal abnormalities. The first patient was a 3 years old girl presenting with chronic renal failure, a renal tubular acidosis type IV, hyperchloremic acidosis, urine concentration anomalies (concentrating power = 120 mosm/kg) and hypercalciuria (calcium/creatinin 1,5 to 3). Renal ultrasound showed bilateral small kidneys (< - 2 SD), and nephrocalcinosis with bilateral lithiasis. The second patient was a 17 year old boy presenting with polyuria and polydipsia, moderate chronic renal failure and urine concentration anomalies (concentrating power = 563 mosm/kg). Ultrasonography showed bilateral kidney hypoplasia (< -2 SD). We review the reported cases with KS and found that kidney malformations occur in 50/272 (18%) of KS. They include horseshoe kidneys, double renal pelvis, duplication of the collecting system, hydronephrosis, megaloureter, ureteropelvic junction obstruction, hydroureter and two cases with severe renal hypoplasia requiring renal transplantation in one case. We conclude that all patients with KS should have functional renal investigation. We propose that functional renal abnormalities could explain in part the post natal short stature in KS and larger studies are necessary to confirm this hypothesis.



**Molecular and clinical evaluation of a boy with Progeroid Variant of Ehlers-Danlos Syndrome. G.**

*Carakushansky<sup>1</sup>, T. Okajima<sup>2</sup>, K. Furukawa<sup>2</sup>, M. G. Ribeiro<sup>1</sup>, M. Carakushansky<sup>3</sup>, E. Kahn<sup>1</sup>.* 1) Dept. Pediatrics, Federal Univ. Rio de Janeiro, RJ, Brazil; 2) Dept. Biochem. II, Nagoya Univ. School of Medicine, Japan; 3) Johns Hopkins Hospital, Pediatric Endocrinology, Baltimore, USA.

Manifestations of progeroid syndrome combined with Ehlers-Danlos (E-D) features have been described in about half-dozen cases registered as a distinct nosologic entity (OMIM 130070). We have studied a 5 year-old boy who exhibited mild mental retardation, short stature, craniofacial disproportion, osteopenia, prominent ears, pectus excavatum, loose and wrinkled skin with increased prominence of veins on hands and feet, bruisability, surgically repaired inguinal hernias, joint hypermobility in digits and subluxation of hips leading to waddling gait. Our patient lacked several characteristic features of progeria such as prominent scalp veins, generalized alopecia, and joint contractures. He also did not conform with well-defined progeroid syndromes such as Hallermann-Streiff, Cockayne and Bartsy syndromes. In 1999, Okajima et al (1) isolated cDNA of the galactosyltransferase I (b4GalT7) presumed to be defective in the progeroid variant of Ehlers-Danlos syndrome. They then searched for mutations in the patient reported by Kresse et al (2) and found 2 changes in the XGPT1 gene. In our patient and in his unaffected parents the genomic sequence of galactosyltransferase I was analysed by direct sequencing of PCR products obtained from amplification of exon segments with primers located in flanking intron or UTR sequences. However, no mutations could be detected except for a single silent heterozygous mutation (R73R, CGC to CGT) in exon 2. This may imply that other related glycosyltransferase is probably mutated in our patient and that further study of its pathogenic role in this disease should be undertaken. All previously reported cases represented isolated cases considered to be the result of autosomal dominant new mutations. Our patient was the product of related and phenotypically normal parents. This fact supports an autosomal recessive mode of inheritance but by itself it is not conclusive. (1) Okajima et al. *J. Biol. Chem.* 1999; 274:22915-22918 (2) Kresse et al. *Am. J. Hum. Genet.* 1987; 41:436-453.

**A Syndrome of Deafness, Neurogenic Muscle Weakness and Optic Atrophy: Rosenberg-Chutorian Syndrome (RCS)? A Disease Looking for a Gene.** *J. Christodoulou*<sup>1, 3</sup>, *L.S. Raffaele*<sup>1, 3</sup>, *B. Bennetts*<sup>1, 3</sup>, *R.A. Ouvrier*<sup>2, 3</sup>. 1) Western Sydney Genetics Program, Royal Alexandra Hospital for Children, Sydney; 2) Neuromuscular Research Institute, Royal Alexandra Hospital for Children, Sydney; 3) Dept of Paediatrics & Child Health, University of Sydney, NSW, AUSTRALIA.

RCS is an X-linked disorder of unknown cause, noted for sensorineural deafness, muscle weakness of neurogenic origin, and progressive optic atrophy, but with preserved intellect. We report here a family in which three affected males may have this disorder, and present our progress to date with molecular studies.

Two brothers, currently aged 5 and 3.5 years, have congenital profound sensorineural deafness, and a peripheral neuropathy with generalised muscle weakness. They are predisposed to acute severe muscle weakness during intercurrent infections, which has led to mechanical ventilation on a number of occasions. More recently the older boy has developed symptomatic optic atrophy. Intellect is probably normal for both. Numerous investigations, including mitochondrial respiratory chain analyses, have been normal. Their parents and sister enjoy normal health, and their hearing is normal. A maternal uncle died of a "muscular dystrophy" at the age of 2 years, and had not developed any speech by the time of death.

Based on these findings we have made a provisional diagnosis of RCS, and have undertaken microsatellite analyses of the X chromosome using the Prism linkage mapping set, Version 2 (Applied Biosystems), using samples from the affected probands, their sister, parents, and maternal grandparents. To date we can exclude about half of the X chromosome, but 4 candidate regions remain to be excluded: a region at Xp11.21, at DXS1214 (~ 13 cM), Xq13.1, bounded by markers DXS986 and DXS8055 (~47cM), a region at Xq25, at DXS1047 (~ 22cM), and Xq28, bounded by DXS1193 and DXS1073 (~ 11cM).

Future studies will include further fine mapping with a denser collection of microsatellites, (including analyses of an unaffected maternal uncle), followed by interrogation of the available genomic databases for candidate gene mutation screening.

**Expression of schizophrenic symptoms in 22q Deletion Syndrome.** *A.S. Bassett<sup>1,2</sup>, S. O'Neill<sup>2</sup>, J. Murphy<sup>2</sup>, P. AbdelMalik<sup>2</sup>, E. Chow<sup>1,2</sup>.* 1) Dept. of Psychiatry, Univ of Toronto, Toronto, ON., Canada; 2) Queen St. Site, CAMH, Toronto, ON., Canada.

**Background:** 22q Deletion Syndrome (22qDS) may represent a genetic subtype of schizophrenia. Individuals with 22qDS are at elevated (~25%) risk for schizophrenia, as are siblings of individuals with schizophrenia (10-15%). At-risk individuals may express mild forms of psychotic or other symptoms. We compared expression of schizophrenic symptoms in two non-psychotic (NP) at-risk groups, 22qDS-NP and siblings from familial schizophrenia (FSZ) families (FSZ-NP), and two affected groups: FSZ and 22qDS-SZ. **Objective:** To determine whether there are phenotypic differences between 22qDS and other genetic forms of schizophrenia. **Method:** A standard scale for severity of schizophrenic symptoms, the Positive and Negative Syndrome Scale (PANSS), was used to assess each subject. Adult subjects with 22qDS-NP (n=15) were compared with FSZ-NP subjects (n=27); subjects with 22qDS-SZ (n=21) were compared with FSZ subjects (n=21); 22qDS groups did not differ significantly on mean age or sex from their respective FSZ group. **Results:** Total PANSS scores did not differ significantly between the two NP groups or between the two SZ groups. However, examination of symptom clusters revealed that 22qDS-NP subjects had more severe negative (e.g., emotional and social withdrawal) and cognitive (e.g., poor abstract thinking and attention) symptom scores than FSZ-NP subjects ( $t=-2.72$ ,  $p=0.01$ ;  $t=-2.08$ ,  $p=0.04$ , respectively). 22qDS-SZ subjects had more severe cognitive and excitement (e.g., poor impulse control, hostility, uncooperativeness) symptom scores than FSZ ( $t=-2.50$ ,  $p=0.02$ ;  $t=-2.35$ ,  $p=0.02$ , respectively). There were no significant differences in the non-psychotic or SZ group comparisons on positive or anxiety/depression symptoms. **Conclusion:** Expression of typical positive and negative symptoms of schizophrenia in 22qDS is similar to that in other genetic forms of schizophrenia, however temper outbursts are more common and cognitive deficits more severe in 22qDS-SZ. Social withdrawal and cognitive deficits may be prevalent in 22qDS without being associated with expression of a psychotic illness.

**Colobomas of the iris and retina in 2 patients with 22q11.2 deletion: more than a deletion?** *K.B. Coleman<sup>1</sup>, K. Kupke<sup>1,2</sup>, K.M. May<sup>3</sup>*. 1) Children's Healthcare Atlanta, Atlanta, GA; 2) Northside Hospital, Atlanta, GA; 3) Dept of Peds, Emory Univ Sch Med, Atlanta, GA.

We describe two infants with iris and chorioretinal colobomas in association with deletion 22q11.2. Case 1 was born at 38 weeks by C-section for fetal distress. The birth weight was 1976 gms, length was 42.5 cms and head circumference was 30.5 cms (all values below -2 SD). The pregnancy was complicated by oligohydramnios, short umbilical cord and IUGR. Clinical findings included bilateral iris and chorioretinal colobomas, abnormal ears, imperforate anus and a very small fistula, multiple small VSDs, mild bilateral hydronephrosis, umbilical and inguinal hernias, GE reflux, tracheal web, intestinal malrotation, hypotonia and developmental delay. Family history was significant for a maternal uncle with colobomas. Case 2 was born by vaginal delivery at 35 weeks. The birth weight was 2240 gms, length was 45 cms, and head circumference was 31 cms (all values at -1 SD). Clinical findings included iris and chorioretinal colobomas and microphthalmos of the left eye, partial iris coloboma of the right eye, pulmonary valve atresia, VSD with overriding aorta and right aortic arch, mild tracheomalacia, deficient upper helix of left ear, bilateral postaxial polydactyly, bilateral deep plantar furrows, low numbers of CD4+ and B cells. In both cases chromosome analysis revealed normal karyotypes while FISH analysis of metaphase chromosomes with the TUPLE1 probe indicated a deletion within 22q11.2. Both cases, but especially Case 1, have features of 22q11.2 deletion as well as features associated with Cat Eye Syndrome, which arises from a duplication of proximal 22q. As both disorders result from structural changes within the same chromosomal region (22q11.2), this may indicate that these cases involve more than a straightforward deletion of the region spanned by the TUPLE1 probe. These two cases expand the phenotype of 22q11.2 deletions and suggest that deletion studies should be considered in patients with colobomas and similar findings.

**Oral, facial, digital, vertebral anomalies with psychomotor delay: an OFD variant or a new syndrome? G.B.**

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The orofacioidigital syndromes (OFDs) are a group of heterogenous hereditary disorders characterized by a spectrum of overlapping clinical findings involving the oral cavity, the face, and the digits. Nine different OFD clinical entities have been recognized, with overlapping clinical findings complicating precise classification. Recently, the first gene involved in the molecular pathogenesis of OFDs has been cloned in Xp22.3, where molecular lesions of *OfdI* have been found segregating with the X-linked dominant form (Type 1) of the disease. Here we report a case of a girl, who presented at birth hypertelorism, micrognathia and a large midline palate cleft associated to an oral hairy polyp. A double labial frenulum and a pedunculated tumor of the tongue completed the clinical presentation. Associated with these oral findings were low set thumbs and clinodactyly of the fifth toe. Chromosomal analysis of the patient showed a normal karyotype. She was re-evaluated at age two and six months, when she presented with psychomotor delay and kyphoscoliosis. Radiographs and CT scan revealed partial synostosis between the atlas and the base of the occipital bone, and multiple proximal-cervical and distal-thoracic vertebral clefts. Magnetic resonance imaging (MRI) of the brain showed a myelinization delay and a smooth empty sella turcica with a negative endocrinological screening for hypopituitarism. The absence of mutations in the coding region of *OfdI* is strong evidence against the diagnosis of OFD Type I, although more complex molecular mechanisms involving this gene in the pathogenesis of the phenotype cannot be excluded. The peculiar synostosis between the atlas and the base of the occiput, are remarkably similar to those described previously by Gabrielli et al. (*Am J Med Genet* 53:290-293), suggesting that these two patients could represent a variant in the wide spectrum of OFDs, or a new unrecognized syndrome.

**Pre- and postaxial polydactyly, abnormal ears, and learning disability: A new dominant syndrome.** *K.M. Boycott, D.R. McLeod.* Dept Medical Genetics, Univ Calgary, Calgary, AB, Canada.

Polydactyly may occur either as a feature of a syndrome or as an isolated defect. Preaxial polydactyly refers to partial or complete extra digits of the radial or fibular ray and postaxial polydactyly of the ulnar or tibial ray. We report a three generation family that presents with pre- and postaxial polydactyly of the hands and feet, abnormal ears, and learning disability. The proband is now 10 months old and was born with preaxial polydactyly of the right foot presenting as a bifid great toe. On the left foot she has postaxial polydactyly type A and syndactyly of the 2nd and 3rd toes. She has type A postaxial polydactyly of the right hand and type B post-axial polydactyly of the left hand. Her mother was born with type B postaxial polydactyly of the right foot, preaxial polydactyly of the left foot, and type B postaxial polydactyly of both hands. The proband's grandmother was born with type B postaxial polydactyly of the left foot. The minor ear abnormalities include thickening and overfolding of the helix in the proband and her grandmother. The proband's mother and grandmother had significant learning difficulties in school, suggesting a learning disability is present.

This family segregates a dominant condition with pre- and postaxial polydactyly, minor ear abnormalities, and learning disability. Both pre- and postaxial polysyndactyly are seen in complex bilateral polysyndactyly, which maps to 7q36, but triphalangeal thumb is not a feature in this family. Pre- and postaxial polydactyly can also be seen in Greig syndrome, caused by mutations in the *GLI3* gene, but the facial features of this syndrome are not seen in this family. There is also some overlap with the syndrome of hallux syndactyly-ulnar polydactyly-abnormal ear lobes (Birth defects 12 (5):255, 1976), but the ear abnormalities are distinct and learning disability was not described. Mutations in human HOX genes have been shown to cause congenital malformations involving the hands and feet, making HOX genes good candidates for the condition segregating in this family.

**Complete Cranio-Fronto-Nasal Syndrome phenotype in a mexican male patient.** *L. Cornejo*<sup>1</sup>, *V. Morán*<sup>2</sup>, *G. Ramírez*<sup>2</sup>, *S. Kawa*<sup>2</sup>, *D. Saavedra*<sup>2</sup>. 1) Sch Medicine, Autonomous Hidalgo State Univ, Pachuca de Soto, Mexico; 2) Dr. Manuel Gea Gonzalez, General Hospital, Mxico, D.F.

**Introduction.** Full spectrum of Cranio-Fronto-Nasal Syndrome (CFNS) is seen mainly in females. Although the responsible gene has been located in X-chromosome and the pedigree of affected families were consistent with X-linked inheritance pattern, the males are very few affected basically in the craniofacial area. The purpose of this paper is to present the first mexican male patient with the complete phenotype of this syndrome including extracranial malformations. **Clinical report.** Propositus is a male twenty-one years old whom mildstones and intelligence were normal. Craniofacial findings are: thick, wiry, and curly hair, low anterior hairline with widows peak; left plagiocephaly, hypertelorism, bifid nose, and wide distance between upper central incisors. The extracranial abnormalities consisting on: thorax asymmetry, S- shaped clavicles abnormally positioned, pectus excavatum, left nipple lower located, left sholder with movement arch limitation; hyperextensible joints, asymmetric lower limb shortness, mild cutaneous syndactyly, grooved nails in hands and feet. A routine kariotype was 46,XY. Craniofacial CTScan confirmed left hemicoronal synostosis, 0-14 cleft, left orbital dystopia, and shortening of the midface length. **Discussion.** Few XY patients have been described world wide with the complete CFNS phenotype. In mexican population where the largest series of this syndrome has already published, none male showed extracranial anomalies. Based on the present data, the previously gene expression proposed as causing this syndrome should be requested.

**Punctata Keratoderma and gastrointestinal malignancy in a Mexican Family.** *N. Davalos*<sup>1,2</sup>, *M. Ruiz*<sup>3</sup>, *A. Garcia-Vargas*<sup>4</sup>, *I.P. Davalos*<sup>1,2</sup>, *D. Garcia-Cruz*<sup>1</sup>, *L.E. Figuera*<sup>1,2</sup>. 1) Div Genetica, CIBO-IMSS, Guadalajara, Mexico; 2) Doctorado en Genetica Humana, CUCS-UdeG, Guadalajara, Mexico; 3) Hospital General de Occidente, SSA, Guadalajara, Mexico; 4) Instituto Dermatologico Jose Barba Rubio, SSA, Guadalajara, Mexico.

The inherited Palmoplantar keratodermas are an extremely heterogeneous group of skin diseases that can be subclassified on the bases of the pattern and age of onset of hyperekeratosis on the palm and sole. In Keratosis Punctata Palmaris et Plantaris (KPPP) type, the lesions are numerous tiny keratotic papules, strictly limited to the volar aspects of the hands and feet, yellow-brown, hard, horny papules, and remain asymptomatic. The disease is inherited as an autosomal dominant trait and the onset is usually in the first decade of life. This appears to be associated with gastrointestinal carcinomas. The prevalence of KPPP in Latin America is less than 0.1%. We describe a Mexican family, with 10 affected individuals through 4 generations, showing KPPP. Clinically the 83 year-old propositus displayed numerous keratotic horny papules of the hands and feet, keratoses progress slowly, asymptomatic, no nail dystrophies associated. Histologically the lesions consist of a marked hyperkeratosis sharply defined, hypergranulosis, without evidence of dyskeratosis or hydropic degeneration in the epidermis (differentiating the condition from porokeratosis), with no neurologic associated features. He presented carcinoma of the pancreas that led to death. The alive remaining affected individuals (4 females and 2 males) only present the skin clinical features. Few other reported cases of hereditary KPPP appear to be associated with gastrointestinal carcinomas, in this family only in the propositus the carcinoma was well documented, it could be explained by the involvement of a regulator gene expressed both in skin and gastrointestinal tract. There have been identified several genes related to palmoplantar keratodermas, hence it is necessary to do linkage analysis in the family presented here in order to determine if any of the known genes is responsible or some other one could be involved.



**Russell-Silver syndrome: A family with autosomal recessive inheritance.** *I. Davalos*<sup>1,2</sup>, *L. Melo*<sup>1,2,3</sup>, *N. Davalos*<sup>1,2</sup>, *L.E. Figuera*<sup>1,2</sup>. 1) Dept Genetica, CIBO-IMSS, Guadalajara, Mexico; 2) Doctorado en Genetica Humana, CUCS-UdeG, Guadalajara, Mexico; 3) Hospital Ortopedico Infantil, Caracas, Venezuela.

The Silver-Russell syndrome (SRS) is an entity with unknown etiology. The main clinical features are intrauterine and postnatal growth retardation, broad forehead with normal head circumference, small triangular shaped face, down turned corners of the mouth, body asymmetry and delayed bone age. The etiology of SRS seems to be heterogeneous, most cases are sporadic although families with dominant or recessive inheritance and X-linked forms have been reported; over 150 cases there were four recurrences in sibs, and in five cases was suggested autosomal dominant inheritance with variable expression. The purpose of this paper is to present the SRS in 2 sisters from unrelated and healthy parents. The proposita was the product of a preterm pregnancy (36 weeks), birth weight of 1,800 gr. On physical examination at 3 months presented low birth weight, retarded growth, craniofacial disproportion, delayed close of anterior fontanelle, triangular face, thin lips, the outer angles of the mouth were down turned, clinodactyly of fifth fingers with delayed psychomotor development and bone age and normal karyotype. These features suggested a SRS. At 8 years old, craniofacial features of SRS and short stature persisted. Case 2. She was the product of a third gestation with a birthweight of 2,200 gr. At physical examination at 6th month revealed 3720g (<3p), a length of 54 cm. (<3p), a head circumference of 40.5 cm (10p), wide anterior fontanelle, prominent forehead, blue sclera, triangular face, small chin, thin lips, body asymmetry, 5th fingers with clinodactyly and delayed bone age. The cases described here presented the principal clinical features of SRS, although etiology remains unclear, the familial cases might be helpful to unveil the etiology of SRS. The fact that autosomal dominant, recessive and X-linked inheritance have been reported, reveals a genetic heterogeneity for SRS. Some molecular studies have shown linkage to chromosomes, 2,7,9,14 and 16, on the other hand, in some cases uniparental disomy of chromosome 7, suggesting that genomic imprinting plays a roll in this entity.

**Rhombencephalosynapsis associated with multiple congenital anomalies: A new syndrome with a probable somatic mutation.** *C.H. Jones<sup>1</sup>, K. Silver<sup>2</sup>.* 1) Dept Pediatrics, Section Gen, Loyola Univ, Maywood, IL; 2) Department of Pediatric Neurology Loyola Univ, Maywood, IL.

Rhombencephalosynapsis is a malformation of the posterior fossa. It is usually confined to the CNS. There are a few reported cases of rhombencephalosynapsis being seen with other congenital anomalies. The anomalies previously described include polydactyly, hypertelorism, low set ears and elongated philtrum. We present a patient with rhombencephalosynapsis in addition to pre-axial polydactyly of the left toes, left tibia longer than the fibula, absent left kidney, left lung hypoplasia, diastinomialia, scoliosis and vertebral anomalies including hemivertebrae. Blood chromosomes at birth were normal 46,XX. Fibroblast chromosomes on the left side of the body were also normal. A head ultrasound performed shortly after birth indicated hydrocephalus. A VP shunt was placed at 10 days of life. At 1 year an MRI of the brain showed absence of the cerebellar vermis with fusion of the cerebellar hemispheres. An MRI of the spine showed diastinomialia in the lower thoracic region at T9-T10. The patient is now 2 years of age. She has developmental delays however is beginning to verbalize and pull to a stand. She is still ventilator dependent at night. We hypothesize that while in some cases rhombencephalosynapsis is an isolated event, it can be part of a syndrome. Our patient had the majority of her other anomalies on the left side of her body. This likely represents a somatic mutation occurring on the left side of her body early in development. A similar mutation in a nonmosaic state would likely be lethal.

**Seckel syndrome: Report of Two Cases.** *N.L. Hatem<sup>1</sup>, S. Tayel<sup>2</sup>, I. Marzouk<sup>1</sup>*. 1) Department of Pediatrics; 2) and Clinical Cytogenetics Unit, Department of Anatomy, Alexandria Faculty of Medicine, Alex, Egypt.

This report describes two Egyptian sibs with Seckel syndrome. Their clinical manifestations included mental retardation, short stature, microcephaly, and characteristic facial appearance in the form of receding forehead, micrognathia, antimongoloid slant of palpebral fissures and large prominent nose i.e. bird-headed dwarfism. One of the two sibs had absent patella. Chromosomal examinations of both demonstrated a high percentage (26% & 31%) of chromosome endoreduplication. Absent patella and chromosome endoreduplication have not been mentioned in Seckel syndrome so warranting its citation. This is the first report from Egypt about Seckel syndrome and the second among arabs.

**Ultrasound Diagnosis of a Sacrococcygeal Teratoma Leading to Prenatal Diagnosis of Jacobsen Syndrome. V.**

*Loik Ramey, F. Bsata, R. Naeem, G.M. Cohn.* Baystate Medical Center, Springfield, MA.

Jacobsen syndrome, also known as 11q- syndrome, is characterized by a clinical phenotype due to a deletion of band 11q23-qter. The phenotype involves IUGR, mental deficiency ranging from moderate to severe, trigonocephaly, epicanthal folds, ocular hypertelorism, short nose with upturned nasal tip, a large, carp-shaped mouth, cardiac defects, and various CNS anomalies. Sacrococcygeal teratomas (SCTs) have not been previously reported in association with Jacobsen syndrome. We report a fetus diagnosed with a SCT at 19 weeks gestation in a 41 year old G5, P2022. Amniocentesis was performed and revealed a 46,XX,del(11)(q23) karyotype consistent with Jacobsen syndrome. Amniotic fluid fetoprotein was normal at 1.21 multiples of the median. The baby was born at 32 1/2 weeks gestation and found to have a coarctation of the aorta, bicuspid aortic valve and a perimembranous ventricular septal defect, and physical features consistent with Jacobsen syndrome. As cardiac surgery was immediately required, removal of the teratoma was delayed until four months of age. Karyotype analysis on tissue taken from the teratoma also revealed the 11q deletion. The literature does indicate that tumors and teratomas may be associated with various chromosome anomalies. Because of the rarity of both conditions, we could speculate that the teratoma may be related to the chromosomal deletion rather than representing an unrelated anomaly.

**Acromegaloid facial appearance (AFA) and generalized congenital hypertrichosis: variant of AFA syndrome or a new syndrome?** C.A. Kim, S.M.M. Sugayama, R.S. Coelho, L.M.J. Albano. Genetics, Dept Pediatrics, Instituto da Crianca, Sao Paulo, Brazil.

AFA syndrome is characterized by acromegaloid facial appearance and thickening of the eyelids, lips and oral mucosa associated with large doughy hands. It was first described in 1985, by Hughes et al., in 13 affected members within four generations. Dallapiccola et al., in 1992, reported more 5 patients in another family and da Silva et al. (1998) and Zelante et al. (2000) reported two other sporadic cases. Irvine et al. (1996) described 4 patients in one family with characteristic acromegaloid facial appearance and generalized hypertrichosis without intraoral lesions. They considered a new entity distinct from AFA syndrome. We studied two sporadic cases with acromegaloid facial appearance with congenital hypertrichosis. The first one, 15 year-old girl, was born with macrosomy and generalized hypertrichosis, including the pubic region. She had a good health and normal psychomotor development and a progressive coarse acromegaloid facial appearance with thickening of the lips. Other distinctive features were skin warty lesions on axilla surface and macrocephaly. Endocrinological evaluation was normal. The second one, 12 year-old girl, was born with normal birth weight and generalized hypertrichosis. She presented mild mental delay and similar coarse acromegaloid facial appearance without macrocephaly. Other distinctive features were arachnoid cyst in the posterior fossa and renal calculosis. Interestingly, arachnoid cyst and skin hemangiomatous lesions were described in association with AFA syndrome, respectively by da Silva et al. (1998) and Zelante et al. (2000). Our patients presented features more similar with that described by Irvine et al (1996): acromegaloid facial appearance and generalized hypertrichosis without intraoral involvement. Further reports are necessary to amplify and better delineate the phenotype characteristics of these rare entities.

**Further delineation of Antley Bixler syndrome.** *K. Okajima<sup>1</sup>, T. Hasegawa<sup>3</sup>, T. Kubota<sup>2</sup>, H. Seki<sup>2</sup>, Y. Sugimoto<sup>2</sup>, Y. Fukushima<sup>2</sup>, N. Yamaguchi<sup>5</sup>, MG. Butler<sup>6</sup>, BP. LeHeup<sup>4</sup>, H. Membre<sup>4</sup>, V. Smith<sup>1</sup>, AJ. Darby<sup>7</sup>, T. Stallmach<sup>8</sup>, A. Schinzel<sup>8</sup>, S. Malcolm<sup>1</sup>, W. Reardon<sup>9</sup>, RM. Winter<sup>1</sup>.* 1) Inst Child Health, London, UK; 2) Shinshu Univ, Matsumoto, Japan; 3) Shizuoka Children Hosp, Shizuoka, Japan; 4) Medical School of Nancy, Nancy, France; 5) Nagoya City Univ, Nagoya, Japan; 6) Children's Mercy Hosp, Kansas City, MO; 7) Robert Jones & Agnes Hunt Hosp, Oswestry, UK; 8) Univ Zurich, Zurich, Switzerland; 9) Our Lady's Hosp for Sick Children, Dublin, Ireland.

Antley-Bixler syndrome (ABS) was first reported as Trapezoidocephaly, midfacial hypoplasia and cartilage abnormalities with multiple synostoses and skeletal fractures. Previous reports suggest that ABS is probably an autosomal recessive condition. FGFR2 mutations and abnormal steroid metabolism are also reported in ABS. We present an evaluation of 14 patients (10 families) whose ethnic background are Asian or Caucasian, who included one of original and consanguineous patients. All patients met the minimal criteria of craniosynostosis, elbow synostosis and femoral bowing and/or fracture. Urogenital involvement was seen 6 patients. One patient had an Arnold Chiari malformation. Six patients survived the neonatal period. DNA was available from 10 patients. No mutations were found in FGFR2 gene. Pathology samples were available from 4 patients. In 3 patients, bone histology revealed that the trabeculae of the primary spongiosa contained occasional cores of cellular hypertrophic cartilage instead of the normal acellular hyaline matrix. Chondrocyte column formation was not disturbed. No microfractures, which may predispose to fracture, were seen. The adrenal glands showed no abnormality. One familial patient had abnormal mucosa outside of the tracheal cartilage. Adrenal steroid profiles were available from 3 patients, and all were within normal limits. These suggest that patients meeting the strict criteria of ABS are not likely to have FGFR2 mutations or an abnormal steroid profile. Patients with FGFR2 mutations and/or an abnormal steroid profile can present with craniosynostosis and elbow synostosis but do not have femoral abnormalities. They most likely represent a phenocopy of ABS.

**Van der Woude syndrome associated with ankyloblepharon filiforme adnatum is not linked to chromosome 1q32-q41 region.** *H. Okur<sup>1</sup>, I. Vargel<sup>2</sup>, F. Ozgur<sup>2</sup>, Y. Erk<sup>2</sup>, A.N. Akarsu<sup>1</sup>*. 1) Gene Mapping Laboratory, Research Center of Ihsan Dogramaci Children's Hospital,; 2) Department of Plastic and Reconstructive Surgery, Hacettepe University, Ankara, Turkey.

Van der Woude syndrome (VWS) is a common form of syndromic cleft lip and palate with typical manifestation of harelip and/or cleft palate, bilateral lower lip pits, hypodontia and an autosomal dominant mode of inheritance with high degree of penetrance. It has already been mapped to chromosome 1q32-q41 between the markers D1S491 and D1S205. No gene has yet been identified. Associations such as ankyloblepharon filiforme adnatum, popliteal pterygium and intraoral tissue bands (syngnathia) have commonly reported with VWS suggesting that these phenotypes might be allelic. Indeed, popliteal pterygium syndrome was further mapped to the same region of interest. We report two multiplex Turkish families with VWS. First family containing 15 affected cases manifested typical characteristics of VWS including Cleft lip (1/15); Cleft palate (5/15); cleft lip and pit (2/15); cleft palate and pit (3/15); cleft lip/palate/pit (4/15). Linkage analysis using the markers D1S245, D1S491, D1S205 and D1S414 provided positive LOD scores. A maximum two point LOD score of 4.438 was observed with D1S205 with no recombination. Thus, we supported locus homogeneity for classical form of VWS. Cleft lip and pit were the characteristic features of the second family containing 15 informative meioses, 5 of whom were affected. In addition to harelip and pit congenital filiform fusion of eyelids, syngnathia were observed in an affected member of the family. One of the affected family members with bilateral cleft lip was recombinant for the entire critical region between cen- D1S1678, D1S245, D1S491, D1S205, D1S414, D1S2141, D1S549-tel. Since this recombination event is observed in a typically affected family member rather than the complicated case we did not expect phenocopy leading to this unexpected result. This result suggests that at least one more locus is responsible for the complicated VWS phenotype.

**PRENATAL ONSET OF INFANTILE CORTICAL HYPEROSTOSIS (CAFFEY DISEASE).** *D. Myles Reid<sup>1</sup>, R. Lachman<sup>2</sup>, G. Seaward<sup>1</sup>, S. Viero<sup>1</sup>, D. Chitayat<sup>1</sup>*. 1) Medical Genetics, Mount Sinai Hospital, Toronto, Canada; 2) The International Skeletal Dysplasia Registry, Los Angeles, California, USA.

Infantile cortical hyperostosis (ICH)(Caffey disease)is a multifocal, inflammatory skeletal process with onset typically within the first few months of life and resolution by 3 years of age. Autosomal dominant inheritance with variable expression and penetrance has been reported. Prenatal onset of ICH is recognized as a distinct phenotype characterized by polyhydramnios, bowed hyperostotic long bones, hydrops, pulmonary hypoplasia and a poor prognosis. Previous case reports have suggested autosomal recessive inheritance. We report a case of prenatal ICH detected at 23 weeks gestation. The mother was a 31-year-old G3P3L0 woman of Jamaican descent and her husband was 32 years old and of the same descent. The couple was healthy, non-consanguineous and their family histories were non-contributory. The couple's first pregnancy was complicated with polyhydramnios and premature rupture of membranes at 21 weeks gestation and resulted in premature delivery and postnatal death. Neither autopsy nor chromosome analysis were completed. The couple's second pregnancy resulted in intrauterine death at 27 weeks gestation. An autopsy showed no obvious abnormalities; however, X-rays were not performed. In the third pregnancy, fetal ultrasounds performed at 12 and 18 weeks gestation reported normal fetal anatomy. At 23 weeks gestation, there was premature rupture of membranes. A repeat ultrasound revealed scalp edema, polyhydramnios, bowed long bones, echogenic gut and bilateral club feet. Postnatal examination of the fetus revealed some rhizomelia and edema of the lower limbs. The right femur appeared short and there was posterior bowing of the right calf. The facial features were mildly dysmorphic with bilateral infraorbital creases and micrognathia. Skeletal X-rays revealed findings consistent with ICH. This case supports previous findings that two distinct forms of cortical hyperostosis exist, infantile and prenatal onset. Regarding the couple's previous pregnancies, X-rays and complete autopsies were not done and thus it is possible that these were also affected with the same condition.



**Further delineation of the 7q36 terminal deletion syndrome.** A. Kwan, A.M. Cherry, I. Galperin, S. Huhn, G.M. Enns. Div Med Genet, Div Cytogenet, Div Neurosurg, Stanford Univ. School of Medicine, Stanford, CA.

The chromosomal region 7q36 contains two homeobox genes involved in the development of the rostral and caudal ends of the neural tube. Mutations in *SHH* cause holoprosencephaly and mutations in *HLXB9* result in the Currarino triad, a pattern of malformation characterized by sacral agenesis (typically a sickle-shaped or "scimitar" hemisacrum), anorectal malformations and a presacral mass. Patients with *HLXB9* mutations have normal intelligence. Isolated terminal deletion of 7q36 has been associated with significant developmental delay, growth retardation, microcephaly, holoprosencephaly and the Currarino triad. In order to further delineate the phenotypic spectrum associated with the 7q36 terminal deletion syndrome, we report the first case of a patient with a *de novo* deletion. One parent carried a balanced translocation in all previous studies of isolated 7q36 deletion that reported parental karyotypes. Our patient presented at 8-1/2 months with microcephaly, mild developmental delay, minor dysmorphic features and chordee. Head MRI and routine karyotype were normal. He developed cellulitis over the sacral area and further investigation revealed an atypical anterior sacral lipomeningocele and a tethered cord. Radiographs showed absence of the sacrum below S2. Because sacral anomalies have been associated with 7q deletions and *HLXB9* maps to the same region, a high resolution blood karyotype was performed with FISH using a 7q subtelomeric probe. A terminal deletion was identified [46,XY,del(7)(q36.2).ish del(7)(q36.3)(TelVysion7q-)]. Parental karyotypes and FISH for the deletion were normal. At 20 months the patient continues to show only mild developmental delay. This case represents a mild expression of the 7q36 terminal deletion syndrome phenotype in a patient with a *de novo* deletion and highlights the utility of combining clinical information and knowledge of the location of specific genes with molecular cytogenetic analysis. We recommend detailed evaluation of chromosome 7q36 in patients with developmental delay, microcephaly and sacral malformations.

**Unusual systemic manifestations in Kabuki syndrome: hypoglycemia, malabsorption, and autoimmune thrombocytopenia and anemia.** *J.E. Ming, K.L. Russell, D.M. McDonald-McGinn, P. Kaplan, E.H. Zackai.* Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine, Philadelphia, PA.

Kabuki syndrome is characterized by postnatal growth retardation, congenital heart disease, developmental delay, skeletal anomalies, and a characteristic facies. We report three unusual systemic manifestations of this condition in our series of 14 patients at The Children's Hospital of Philadelphia: neonatal hypoglycemia, intestinal malabsorption, and autoimmune thrombocytopenia and anemia. Neonatal hypoglycemia was present in three patients in our series. Two patients presented on the first day of life with severe hypoglycemia, one of whom had hyperinsulinism. The third patient was noted to have hypoglycemia at age 11 months. Severe intestinal malabsorption has been noted in two of the patients we have evaluated. The first patient presented with malabsorption at age 4 months and was given pancreatic enzyme supplementation. The second patient presented at age 9 years with severe watery diarrhea and had decreased fat, carbohydrate, and protein absorption. Parenteral nutrition was required. Two patients have presented with a combination of autoimmune thrombocytopenia and hemolytic anemia. At age 4 years, the first patient was noted to have pancytopenia. Autoantibodies were present and he responded to steroid treatment. The second patient presented at age 13 years with thrombocytopenia. Anti-platelet antibodies were detected, and the peripheral blood smear was consistent with hemolytic anemia. The three serious medical conditions described in these patients are rare complications of Kabuki syndrome and have been occasionally noted in previous reports. Our experience suggests that these findings may occur at a greater frequency than previously recognized. In addition, as these diseases will bring a child to medical attention and could prompt a clinical genetics evaluation, recognition that these findings are associated with Kabuki syndrome may aid in diagnosis.

**Shprintzen-Goldberg syndrome and cervicomedullary compression.** *H. Kawame<sup>1</sup>, H. Shigeta<sup>2</sup>, S. Sakazume<sup>1</sup>, Y. Fukushima<sup>1</sup>.* 1) Div of Clinical and Molecular Genetics, Shinshu Univ Schl of Med, Nagano; 2) Div of Neurosurgery, Nagano Children's Hospital, Nagano, Japan.

Shprintzen-Goldberg syndrome (SGS) is a rare disorder associated with a complex phenotype of variable craniosynostosis, developmental delay, characteristic facial features, and other skeletal and connective tissue anomalies. At least 20 patients have been reported in the literature, the phenotypic spectrum of SGS are not well delineated. We report a 15 month-old female patient with SGS and stenosis of the foramen magnum with cervicomedullary compression.

The patient was the first child born to unrelated, healthy patients. At age of one month, strabismus was noted and ophthalmologic examination revealed exudative vitreoretinopathy. At 4 months, mild developmental delay was noted. At 12 months, MRI revealed stenosis at the level of the foramen magnum and cervicomedullary compression. The compression was secondary due to hypoplasia of the posterior arch of C1. On physical examination at 15 months, she had dolicocephaly, ocular proptosis, hyperterolism, high arched palate, pectus excavatum, and arachnodactyly of the fingers and toes. She was mildly delayed. She had general hypotonia, joint hyperextensibility, especially in the knee and ankle joints. Her deep tendon reflex was not brisk. She had high myopia. A 3D CT scan reveal no evidence of premature closure of cranial sutures, but skull Xp showed elevation of the lesser wing of the sphenoid bone, which was the characteristic sign for craniosynostosis.

This case demonstrates important clinical aspect of SGS. Although cervical vertebral anomalies were described in a few patients with SGS previously, those manifestations were not emphasized. Clinicians should be aware of the craniovertebral junction abnormalities in SGS. Careful evaluation of cervicomedullary region, including the sagittal MRI study, is recommended in all patients with SGS. Exudative vitreoretinopathy was reported only once. Ophthalmologic examination is should be offered to infants with SGS.

**Further evidence for the Hall-Hittner syndrome: A case report.** *T. Jewett, L. Terry.* Dept Pediatrics, Medical Gen, Wake Forest Univ Sch Medicine, Winston-Salem, NC.

CHARGE association is a non-random cluster of congenital anomalies (ocular Coloboma, Heart malformation, choanal Atresia, Retardation of growth and/or development, Genital hypoplasia, Ear anomalies with/without hearing loss) which occurs together more often than expected by chance. Historically, inclusion criteria for diagnosis include: 1) either coloboma or choanal atresia, and 2) four of the six features listed in the CHARGE acronym. Because no single, unifying etiology has been determined, CHARGE has been considered an "association" rather than a "syndrome." Several papers have now been published that have broadened the phenotype, and a significant percentage of affected individuals have been found to have partial semicircular canal hypoplasia/aplasia with or without Mondini malformation. Amiel et al recently described three individuals who had three of the four diagnostic criteria for CHARGE association without coloboma or choanal atresia; all three had semicircular canal agenesis or hypoplasia. They proposed that temporal bone malformations be included as a major criterion for diagnosis and that CHARGE is a specific enough entity to be designated a "syndrome." Graham agrees that a specific, recognizable pattern has emerged among individuals with CHARGE association and proposes that this entity be called the Hall-Hittner syndrome. We report an infant who has three out of four diagnostic criteria for CHARGE association as well as Mondini malformation and dysplasia and/or absence of the semicircular canals bilaterally, adding further evidence to support the Hall-Hittner syndrome as a diagnostic entity.

J.W. was born at 36 weeks to a 33 y.o. G2 P0 SAB1 woman. Soon after birth, he was diagnosed with tracheoesophageal fistula. In addition to the above temporal bone anomalies, he has dysplastic ears, profound sensorineural hearing loss, large secundum atrial septal defect, right facial nerve palsy, and suboptimal growth despite gastrostomy tube placement. Chromosome analysis and FISH for 22q11 deletion are normal.

**New autosomal recessive syndrome resembling Nager syndrome.** *S.J. Kennedy<sup>1,2</sup>, A.S. Teebi<sup>1,2</sup>*. 1) Clinical Genetics, Hospital Sick Children, Toronto, ON, Canada; 2) University of Toronto.

Nager syndrome is an autosomal dominant condition associating mandibulofacial dysostosis and limb anomalies. Individuals with this syndrome typically have malar hypoplasia, ear anomalies, conductive hearing loss, micrognathia, hypoplastic/absent thumbs, radial hypoplasia, and radio-ulnar synostosis. An autosomal recessive form of Nager syndrome may exist based on reports of affected sibs with unaffected parents. We report on sibs, with manifestations resembling Nager syndrome. The parents are a nonconsanguineous phenotypically normal couple of Punjabi descent from an isolated religious community. There is no family history of individuals with a similar medical and developmental problems. The proband has microcephaly, sloping forehead, malar hypoplasia, upper eyelid ptosis, long eyebrows, short and downward slanting palpebral fissures, a large beaked nose, bilateral cleft lip and palate, micrognathia, bilateral microtia and moderate to severe conductive hearing loss. In addition, she showed bilateral hypoplasia of the thumb and thenar eminence. X-rays of the hands revealed bilateral fusions of the capitus and hamate, and short 1<sup>st</sup> and 5<sup>th</sup> metacarpal bones. X-rays of the feet demonstrated bilateral shortness of the 1<sup>st</sup> metacarpal bone and an additional sesmoid bone in the distal portion of the carpal bone on the left. The left talus and lateral cuneiform bone are fused. The proband is mildly developmentally delayed. Chromosome analysis on the proband, including FISH and SKY for cryptic chromosomal rearrangement detection, was normal. The proband's younger brother was born with unilateral cleft lip and palate, beaked nose, bilateral upper eyelid ptosis, bilateral microtia and has moderate to severe conductive hearing loss. Although the spectrum of anomalies in these sibs includes those seen in patients with Nager syndrome, the presence of microcephaly, cleft lip and palate, the peculiar beaked nose, blepharophimosis, ptosis of the upper eye lids, microtia, symmetrical involvement of the thumbs and big toes and developmental delay makes it a distinct entity likely inherited in an autosomal recessive manner.

**A Patient with Supernumerary Nostril and Other Anomalies.** *J. Huber, J.B. Volpon, E.S. Ramos.* Medicine School of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

Nose duplication includes polirinia, double nose, and supernumerary nostril. It is an extremely rare congenital anomaly with few reports in the literature. The descriptions include only sporadic cases and rarely associated with other anomalies like naso-ocular cleft. Some authors suggest that the duplicate nose results from the evolution of four nasal pits in the embryological development of the face and the supernumerary nostril results from an accessory nasal pit located laterally to the nasal laminae, not interfering with their fusion and the formation of the one nasal septum. The objective of the present work is to report a female patient with a supernumerary nostril associated with choanal atresia, coloboma of the right lower eyelid, external genitally hypoplasia and limbs anomalies that include fifth fingers clinodactily, genu varum and knees joints with restriction movements, club feet with a syndactily between the second and third toes. An axial computed tomography scan showed an asymmetry of maxilla sinuses, larger on the left side, with normal aeration; nasal septum dislocation; malformation of the right nasal cavity with choanal atresia; a third nostril lateral of the left nostril without communication with the nasal cavities. X-ray of the hip showed asymmetry of the proximal femora epiphyses which the smaller on the right side. There is a patient reported in the literature with a supernumerary nostril, bilateral choanal atresia, microphthalmia, coloboma of the iris and a total ablatio of the retina in the right eye. The patient was diagnosed as CHARGE association. Our patient does not fulfill the diagnostic criterias for CHARGE syndrome. Furthermore the patient has limb anomalies that are rare in CHARGE syndrome. As she has not all criteria for this syndrome we believe that the set of malformations may be represents a new syndrome. Supported by: FAEPA, CAPES.

**SHPRINTZEN-GOLDBERG SYNDROME IN A FAMILY WITH AUTOSOMAL DOMINANT TRANSMISSION AND VARIABLE EXPRESSIVITY.** *V.L.G.S. Lopes, J. Zimmermann, D.Y.J. Norato.*

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The Shprintzen-Goldberg syndrome (SGS) is mainly characterized by craniosynostosis, marfanoid habitus and skeletal abnormalities. Mental retardation is variable. Mutations on FBN1 gene (15q21.1) have been described in some cases. Dominant pattern of transmission is suggested, but familial cases with male-to-male transmission have not been reported before. A similar picture with normal intelligence has been considered a different entity (Marfanoid habitus, craniosynostosis and normal intelligence - MHCNI) We describe a family presenting variable features of SGS in 4 individuals, a father and 3 children. The propositus is a 13 year-old boy, referred to us for evaluation for mental retardation, premature fusion of sutures and dysmorphic findings. On physical examination, we observed flattened occiput, low set and dysmorphic ears, facial asymmetry, widened frontal, ocular hypertelorism, downslanting palpebral fissures, prominent eyes, malar hypoplasia, beaked nose, shortened philtrum, thick lips, high and narrow palate, absence of upper lateral incisors, widened spaced nipples, scoliosis, dolichostenomelia and arachnodactyly. His father presented downslanting palpebral fissures, beaked nosed, pectus carinatum, scoliosis and dolichostenomelia. Ophthalmological evaluation detected severe myopia in the father and the propositus. Chromosomal analysis of the propositus and of his father were normal (46,XY). His two sisters also presented some characteristics, such as widened frontal, dysmorphic ears, high and narrow palate, pectus excavatum, widened placed and hypoplastic nipples, scoliosis, dolichostenomelia, and arachnodactyly, with normal intelligence. Cardiological evaluation of all dolichostenomelia, arachnodactyly the individuals were normal. This family would confirm dominant pattern of transmission with variable expressivity. It would also suggest that SGS and MHCNI could be extremes of a same condition.

**Congenital laryngotracheal webs and early onset colorectal carcinoma in a family with three-generation transmission of del(22q11.2).** *J. Larsen-Haidle, K. Keppler-Noreuil, A.B. Kanis, A. Muilenburg, J. Welch, Q. Qian, L. Yang, S. Patil.* Pediatrics, Univ Iowa, Iowa City, IA.

Microdeletion of chromosome 22q11.2 has a wide phenotypic spectrum with variable associated anomalies. Familial transmission of this deletion in two-generations has been reported previously. To our knowledge, we report, the first three-generation transmission of del(22q11.2) in one family who had unique findings of congenital laryngotracheal webs (CLW) and early onset colorectal carcinoma.

The proband, a 53 year-old WF, presented with psychiatric illness and mild mental retardation. As a newborn, she was diagnosed with a VSD, and severe respiratory problems secondary to a CLW. She was diagnosed with sigmoid colon adenocarcinoma at 41 years. Her daughter presented with apparent hypertelorism; while her grandson had a laryngotracheal web, hoarse voice, hypertelorism, VSD, and anal stenosis. All three individuals had del(22q11.2).

CLW are rare defects that may occur alone or in association with other anomalies. Two families with CLW, congenital heart disease, and short stature have been reported; however, analysis for del(22q11.2) was not performed. These findings suggest that CLW may be caused by the deletion. The early onset of colorectal malignancy in our case is of interest. Recently, an unidentified putative tumor suppressor gene involved in colorectal carcinoma has been localized to 22q. The loss of heterozygosity for alleles on certain chromosomes, including chromosome 22q, has been identified in nearly 50% of colorectal tumors. Further studies are needed to assess whether individuals with del(22q11.2) are at increased risk for developing this malignancy.



**Unusual GI pathology in a patient with Rett Syndrome (RS).** *K.A. Washington<sup>1</sup>, E.J. Hoffenberg<sup>2</sup>, C.-H. Tsai<sup>3</sup>, E.R. Elias<sup>1,3</sup>.* 1) Pediatrics, Children's Hospital, Denver, CO; 2) Department of Gastroenterology, Children's Hospital, Denver, CO; 3) Department of Genetics, Children's Hospital, Denver, CO.

Rett Syndrome (RS), an X-linked neurodegenerative disorder with progressive encephalopathy, was recently found to be caused by a mutation in the gene coding for production of methyl-CpG-binding protein 2 (MECP2). Girls with RS typically present with normal growth and development for the first six to eighteen months of age, then show a slow deterioration.

Patients with RS frequently present with gastrointestinal complaints, among which constipation is common. We would like to present an RS patient with very unusual gastrointestinal pathology. This 10-year-old girl with a typical neurodevelopmental course for RS, and a documented missense mutation in MECP2 (T158M, felt to be a disease-causing mutation which has been identified in many other RS patients), presented with symptoms suggestive of colitis, consisting of constipation, decreased appetite, and noticeable hematochezia. At colonoscopy, submucosal polypoid lesions were present throughout the left colon. Pathology review suggested pneumatosis cystoides intestinalis (PCI), a rare condition in pediatrics, associated with constipation. The patient's symptoms continued despite prednisone, but improved upon withdrawal of lactulose and aggressive treatment of constipation. An extensive literature search revealed no prior association between RS and PCI.

This case illustrates that bowel problems may be associated with RS, perhaps due to an effect of an MECP2 mutation on intestinal function. To further evaluate this hypothesis, and to determine if there is any genotype/phenotype correlation between specific mutations and clinical manifestations, it will be important to document the frequency and types of intestinal disorders found in RS patients with confirmed MECP2 mutations.

**Hidrotic Ectodermal Dysplasia with lacrimal duct stenosis in a mother and daughter: New disorder or variant of a known disorder?** . *M.S. Williams<sup>1</sup>, K.D. Josephson<sup>2</sup>*. 1) Dept Pediatrics, Gundersen Lutheran Medical Ctr, La Crosse, WI; 2) La Crosse Regional Genetic Services Project, La Crosse, WI.

A child was referred for evaluation of brittle hair and nasolacrimal duct obstruction. Family history suggested that the mother was also affected, but no other affected family was identified. There was no family history of clefting, limb anomalies or other features suggestive of an ectodermal dysplasia. The patients were found to have an hidrotic ectodermal dysplasia (ED) with nasolacrimal duct obstruction. The ED involved the scalp hair permanent teeth but not deciduous teeth, and nails. Both individuals had normal sweating by report. The child at age 4 still had significant nasolacrimal duct stenosis. The mother had epiphora despite two probes of the tear ducts. No other unusual features were seen in either individual. Inheritance is compatible with either Autosomal Dominant (AD) or X-linked with mild carrier expression.

Over 150 forms of ED have been clinically described. The patients do not clearly fit into any of the known AD or X-linked EDs. Clinical features match those of a Lebanese kindred with a presumed Autosomal Recessive (AR) hidrotic ED (OMIM 602401). It is possible the disorders may be allelic. Alternatively, this family could represent a variant of X-linked anhidrotic ED (OMIM 305100) or Ectodermal Dysplasia, Ectrodactyly and Clefting syndrome (EEC3, OMIM 604292). EEC3 is caused by mutations in the p63 gene. Lacrimal duct obstruction is common in EEC syndrome. Families with p63 mutations have also been reported with two of the three cardinal features, as well as isolated clefting and ectrodactyly. A pure ED associated with a p63 mutation has not been reported. Nonetheless, given the range of conditions associated with p63 mutations combined with the presence of nasolacrimal duct obstruction it seems a reasonable candidate gene for this family. Mutation analysis will be undertaken.

We suspect that this family represents a hitherto unrecognized variant of a known ED syndrome rather than a new syndrome.

**Homozygosity for a premutation of the FMR1 gene and normal phenotype in two sisters from a family with fragile X syndrome.** *H. Thiele*<sup>1</sup>, *H. Peters*<sup>2</sup>, *D. Bahrke*<sup>3</sup>, *I. Hansmann*<sup>1</sup>. 1) Institut fuer Humangenetik und Medizinische Biologie, Martin-Luther Universitaet, Halle/S; 2) Medizinische Genetik, Charité, Berlin; 3) St. Barbara Krankenhaus, Halle/S, Germany.

Fragile X syndrome is one of the most common causes of mental retardation due to an expansion of a CGG repeat motif at the 5' UTR of the FMR1 gene during transmission of a premutation in a heterozygous female. According to our knowledge no female with homozygosity for the premutation and only 3 females with compound heterozygosity (full mutation on one X chromosome and premutation on the other X chromosome) have been described so far (Mila M et al. 1996; Russo S et al. 1998; Linden MG et al. 1999). We report on an unusual family with fragile X syndrome in a boy and homozygosity for a premutation in his mother and his aunt. The 4-year-old boy was born after an uneventful pregnancy, birth weight was 3050g and length 49cm. At an age of 2 years he displayed slight dysmorphic facial features, behavioral problems, a retarded speech and language performance. Southern blot analysis revealed a full mutation with a complete methylation of the EagI-restriction site at the 5' UTR of the FMR1 gene. Analysis of his 25-year-old mother revealed a premutation in the range of 70-130 CGGs on one X chromosome and a further premutation of 54 CGGs on the second X chromosome. Her 21-year-old sister was shown to have 2 premutations as well but in the range of 100-200 repeats. Both sisters do not show any sign of intellectual impairment, motor retardation, speech problems, dysmorphic features or irregularities of menstrual cycles. This demonstrates for the first time that homozygosity for a FMR1 premutation remains without clinical consequences for carriers. Southern blotting of the clinically normal parents revealed a premutation of approx. 200 repeats in the father and of approx. 100 repeats in the heterozygous mother. Their maternal grandmother was shown to have together with a normal allele mosaicism for a full mutation and a premutation. Apparently, both daughters inherited a contracted paternal premutation. There is no evidence for consanguinity in this unusual family.

**Severe cases of femoral hypoplasia-unusual facies syndrome may have increased risk for airway obstruction.**

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Femoral hypoplasia-unusual facies syndrome (FHUFS) is a heterogeneous disorder. Features include short femurs, hearing loss, cleft palate, and micrognathia. We report on 2 severe FHUFS patients with significant airway obstruction, which has not been previously described. Close supervision of the airways of FHUFS patients should be considered to avoid potentially lethal complications.

Case 1: Patient 1 was the product of a twin gestation born to a woman with unrecognized insulin-dependent diabetes until 16 weeks. Prenatal sonograms showed short femurs and a hypoplastic sacrum thought to be due to caudal regression. Delivery at 36 weeks gestation was via C-section for breech presentation. This infant had respiratory distress requiring intubation for the first 24 hours. Post-extubation he had severe stridor requiring re-intubation. He was extubated gradually the second time and did well. Since then he has had multiple episodes of stridor and has been diagnosed with right vocal cord paralysis. In addition, he had a recurrent left supraglottic saccular cyst, which resolved post surgery. Other problems include hypoplastic femurs, club feet, cleft palate, micrognathia, cryptorchidism, small kidneys, and partial sacral agenesis. His twin sister had no anomalies.

Case 2: Patient 2 was the product of an uncontrolled insulin-dependent diabetic. Prenatal sonograms suggested caudal regression. The infant was delivered at 36 weeks' and had respiratory distress, resulting in a difficult intubation. At 5 years, she still requires a tracheostomy tube and intermittent ventilator support at night. The cause of her airway compromise is thought to be due to micrognathia and severe tracheomalacia. She has had multiple hospitalizations for respiratory distress. Other problems include absence of her right leg, severe shortening of the left femur, hypoplasia of the lower leg, sacral agenesis, unilateral renal agenesis, camptodactyly, cleft palate, micrognathia, and mixed hearing loss.

**Autistic disorder in Sotos overgrowth syndrome.** *S. Sigurdardottir*<sup>1</sup>, *K. Kristjansson*<sup>2</sup>. 1) State Diagnostic & Counseling Center, Kopavogur, Iceland; 2) Department of Pediatrics, University Hospital of Iceland, Reykjavik, Iceland.

Sotos syndrome is a rare nonprogressive neurological condition of an unknown etiology. Behavioral patterns that frequently have been described in patients with Sotos syndrome include tantrums, destructiveness, eating and sleeping difficulties, stereotypic behavior and social withdrawal. Of the approximately 300 cases identified only one had associated autism. We describe a patient with Sotos syndrome and autistic disorder. He was a full term infant. Head circumference at birth and at the age of three was above the 98th percentile. Facial features were characteristic for Sotos syndrome, including a prominent forehead and prognathism. Cerebral gigantism was confirmed with MRI of the brain which in addition showed occipital polymicrogyria. Bone age at the age of three was slightly delayed. He had severe hypotonia in infancy with delayed motor and language milestones. The diagnosis of autism was based on results of The Autism Diagnostic Interview (ADI-R) at the age of three years and eight months and The Childhood Autism Rating Scale (CARS). Verbal IQ on the WPPSI-R at the age of five was 65 and performance IQ was 106. Although most cases of Sotos syndrome have advanced bone age and mental retardation those are not strict diagnostic criteria for this syndrome. This case adds to the description of the behavioral phenotype associated with Sotos syndrome where social withdrawal and stereotypic behaviors are prominent. These features were present in our patient to the degree that diagnostic criteria for autistic disorder were reached. Quantification of behavioral symptoms has clinical implications both for medical and behavioral treatment as well as in family counseling.

**Siblings with multiple anomalies: Crane Heise vs. new syndrome?** *D.J. Zand<sup>1</sup>, D. Huff<sup>2</sup>, D. Carpentieri<sup>2</sup>, N. Spinner<sup>1</sup>, E. Zackai<sup>1</sup>.* 1) Div of Human Genet and Mol Biol.; 2) Dept of Path, The Children's Hospital of Philadelphia, Philadelphia, PA.

We present 2 sibling fetuses with a severe phenotype. Pregnancies were electively terminated due to ultrasound identification of multiple anomalies. Both were SGA and showed absence or hypoplasia of frontal, parietal, and occipital bones. They also showed absence of hard and soft palates. Both had severe micrognathia and hypertelorism. One had absence of cervical vertebral bodies and shortened clavicles. Both showed absent middle phalanges of hands and feet, clubfeet and multiple contractures. One showed a congenital heart defect consisting of thickened walls of ascending aorta and pulmonary artery. Brain pathology for one noted agenesis of the corpus callosum and cingulate gyrus. One fetus demonstrated absence of body and scalp hair, anisospenia, and redundant loops of bowel with aberrant attachments. High-resolution karyotype on both blood and amniotic fluid cells was 46,XY (fetus 1) and 46,XX (fetus 2). FISH probes for 22q11.2 deletion, Williams syndrome, and 18p were all normal (fetus 2). Parental telomeric analyses were normal. Crane and Heise (*Pediatrics* 68: 235-237, 1981) described three SGA sibling fetuses (2 male, 1 female) with severe developmental anomalies, including: poorly mineralized calvarium, dysmorphic facies with associated cleft palate, micrognathia, upturned nares, and apparent ocular hypertelorism, and musculoskeletal anomalies including absence of cervical vertebrae and clavicles, clubfeet, and soft tissue syndactaly. Autosomal recessive inheritance was presumed. Our cases were also SGA and showed poor mineralization in one or more cranial bones, absence of cervical vertebral bodies, agenesis of hard and soft palates, severe micrognathia, hypertelorism, clubfeet. One case had shortened clavicles while the other had agenesis of the corpus callosum. In contrast, these fetuses have hypoplasia or absence of middle phalanges of both hands and feet without soft tissue syndactaly, structural cardiac defects, as well as other internal organ anomalies. These differences may represent either a phenotypic variation of Crane Heise Syndrome or a new syndrome.

**Optic Atrophy, severe epilepsy and mental retardation: A novel autosomal dominant disorder in a large family.**

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Although optic atrophy, seizures and mental retardation can be seen as components of a number of disorders of varying etiology including mitochondrial defects, they are not reported to occur in combination as a single gene disorder with high penetrance. We report here a six-generation family segregating optic atrophy, severe seizures with frequent status epilepticus in adolescence, and variable mental retardation; inheritance appears to be autosomal dominant with high penetrance. The family includes individuals across six generations, and the number of affected members ( from family history, medical records and clinical evaluations since 1980) is about 18. Most affected members have progressive optic atrophy of insidious onset leading to significant visual impairment in many and blindness in at least three individuals. The age of onset of the visual symptoms has been variable with earliest diagnosis at 11 years of age. Seizures of a tonic-clonic nature affects almost all the individuals with optic atrophy. Episodes of status epilepticus in some of the affecteds has required constant medication and aggressive management. In two individuals with adolescent onset seizures, progressive increase in severity and refractoriness to medical therapy led to surgical hemispherectomy. Mental retardation of a mild to moderate degree is present in a number of affecteds but is not a constant association. Initial simulation studies indicate a maximum achievable LOD score of 5.331 with a mean of 2.578 (at  $q=0.05$ ). Efforts are underway to identify linkage by a whole genome scan using markers spaced at 10 cM intervals. To our knowledge this unique family represents a new autosomal dominant optic atrophy syndrome associated with severe epilepsy and mental retardation.

**Severe growth retardation in a patient with a deletion of the short arm of chromosome 3: A case study.** *J. Van Brunt<sup>1</sup>, H. Andersson<sup>1, 2</sup>, M.M. Li<sup>1, 2</sup>.* 1) Hayward Genetics Center, Tulane University School of Medicine, New Orleans, LA; 2) Department of Pediatrics, Tulane University School of Medicine, New Orleans, LA.

Deletion of the distal portion of the short arm of chromosome 3 is a rare occurrence. To our knowledge, only 34 cases have been reported in the literature since the first description of this syndrome by Verjall and DeNef in 1978. Due to its variable phenotypic expression, it is assumed that the 3p- syndrome represents a contiguous gene syndrome with an undefined number of genes contributing to the phenotype. The most common phenotypic expressions that characterize the syndrome include intrauterine and postnatal growth retardation, psychomotor retardation, craniofacial anomalies, skeletal anomalies, congenital heart disease, urinary tract anomalies, and renal anomalies. We describe a newborn patient with a de novo deletion of 3p25.1@3pter and compare the clinical manifestations with a review of clinical features from 34 previously described cases. Our patient was female and born to a 14-year-old mother (gravida 1 para 1) and a 16-year-old father. Physical examination revealed some commonly found craniofacial anomalies including ptosis of the eyelids, microcephaly, long and flat philtrum, displastic ears with a ear pit on the left ear. She had severe, proportionate, growth retardation. The patient also manifested hypoplastic fingers and toes with absent nail beds, which have not been previously reported. This raises the possibility that there is a gene or genes located in this region with responsibility for digital development. The deletion may cause either haploinsufficiency of the gene (autosomal dominant trait), or loss of heterozygosity if the gene on the normal homologous chromosome was mutated (autosomal recessive trait). Most of the current research on 3p- syndrome has been directed at identifying genes that map in the deleted region and connecting their developmental function with specific phenotypic expressions of the syndrome. As more information about this rare syndrome is collected, a better phenotype-genotype correlation will evolve.



**Atypical presentations of Bloom syndrome.** *H. Zhao, R.J. Hopkin, H.M. Saal.* Division of Human Genetics, Children's Hosp Medical Center, Cincinnati, OH.

Bloom syndrome is a rare autosomal recessive condition caused by impaired DNA repair. Characteristic findings include congenital dwarfism, sun-sensitive telangiectatic rash, hypo- and hyperpigmented skin lesions, chromosome instability, and predisposition to malignancy. A significant number of affected individuals are of Ashkenazi Jewish ancestry, suggestive of a founder effect. We have evaluated 5 new non-Jewish patients with Bloom syndrome, all diagnosed by history of pre- and postnatal growth deficiency and microcephaly. All cases were confirmed by demonstration of increased sister chromatid exchange. No patient had a history of skin rashes or photosensitivity. Additional unexpected features include mental retardation, intestinal malrotation with intussusception, kyphosis, hypoplastic thumbs, absent ovaries, iris and retinal coloboma, glucose intolerance, and idiopathic pancreatitis. Hypoplastic thumbs in one case led to an initial erroneous clinical diagnosis of Fanconi anemia; however, DEB stimulated chromosome breakage analysis was normal. Of interest is that 3 of our patients are of Appalachian ancestry and 2 (male and female siblings) are of German ancestry. These findings are suggestive of a specific mutation with founder effect. Molecular studies are currently being pursued.

In Bloom syndrome, the presence of growth deficiency and microcephaly may be the only presenting signs. Therefore, this condition may exist in the absence of typical skin findings. Furthermore, the presence of other atypical malformations, such as radial anomalies and intestinal malrotation do not necessarily exclude Bloom syndrome as a diagnosis.

**Reynolds Acrofacial Dysostosis: Report of an Additional Family.** *A.R. Turner, D. Conrad, W. McGuirt, V. Shashi.* Department of Pediatrics, Section on Medical Genetics, Wake Forest University School of Medicine, Winston-Salem, NC.

Since the association between acral anomalies and mandibulofacial dysostosis was first described, the acrofacial dysostoses (AFDs) have been defined as a heterogeneous symptom complex. Reynolds described an Idaho family with AFD of unique presentation in 1986. Reynolds AFD is distinguished by consistent craniofacial findings, variable manifestations of acral anomalies in the radial ray, normally appearing ears, and mild congenital hearing loss. We present the second reported family with Reynolds acrofacial dysostosis. The proband was brought to our attention at 19 months, due to unusual facial features. His examination revealed all of the hallmark craniofacial features, including ptosis, hypertelorism, malar hypoplasia, and micrognathia. The ears were simple and normally set. Radiographs of the hands confirmed bilateral metacarpal shortening. Audiometry revealed no evidence for hearing loss. His development has been normal throughout four years of follow up. The proband's mother and his five year old maternal half sister have mild to moderate conductive hearing loss, normal intelligence, and both share the proband's collection of physical features. The proband has an additional maternal half sister and a half brother who have no features suggestive of acrofacial dysostosis. Pedigree analysis of the immediate family suggests dominant inheritance, with minimal variability among affecteds. The proband's mother reports a paternal first cousin once removed with marked ptosis and a facial appearance suggestive of Reynolds AFD. The intervening paternal relatives are reported to be free of any outward features of Reynolds AFD. Because extended family members may have subtle features of Reynolds AFD that would not bring them to the attention of a medical geneticist, we cannot fully evaluate the possibility of variable penetrance and expressivity. However, this family provides the first suggestion of broadly reduced penetrance and expressivity of Reynolds AFD within a single family. Additional investigation of this intriguing possibility may ultimately lead to a better understanding of this very rare form of AFD.

**Pallister-Killian Syndrome: A study of three unrelated Arab Families.** *L.A. BASTAKI, KAMAL.K NAGUIB, SADIKA.A. AL-AWADI, SAYED.A. GOUDA.* Maternity Hosp, Kuwait Medical Genetic Ctr, Khaldiya, Kuwait.

Here in, we are reporting three unrelated Arab families with Pallister-Killian Syndrome in order to highlight the clinicogenetic heterogeneity of this rare syndrome. They are presented with severe global developmental delay, Normal birth weight, hypotonia, craniofacial dysmorphism & hypopigmentation involving mainly the face. The constant craniofacial features were wide anterior fontanel, frontal and partial bossing and bolding flat nasal bridge, prominent philtrum and low set posterior rotated ears. Chromosomal study using peripheral blood was normal while fibroblast culture revealed super numerrary isochromosome for the short arm of chromosome 12 in two third of the examined cells. The pathogenesis, clinical picture, genetic counselling and prognosis are discussed. Key word: Pallister-Killian syndrome, isochromosome 12p, fibroblast culture, mosaic tetrasomy 12p, prenatal diagnosis.

**Prenatal and postnatal multiple congenital abnormalities in a girl with a telomeric X;13 unbalanced**

**translocation.** *I.M. Gaspar<sup>1</sup>, C.F. Pinto<sup>1</sup>, A.J. Macedo<sup>2</sup>, S. Azevedo<sup>3</sup>, J. Guimaraes<sup>3</sup>, L. Sampaio<sup>3</sup>, M. Goncalves<sup>3</sup>, J. Correia<sup>4</sup>, A. Ferreira<sup>4</sup>, M.J. Feijoo<sup>1</sup>.* 1) Medical Genetics Dept, Egas Moniz Hosp, Portugal; 2) Dept of Pediatric Cardiology, Sta Marta Hosp, Portugal; 3) Clinic University of Pediatric, Sta Maria Hosp, Portugal; 4) Dept of Obstetrics, Alfredo Costa Maternity Hosp, Portugal.

A 25 year old primigravida was referred for amniocentesis due to multiple congenital anomalies identified in her fetus by an obstetric ultrasound at 24 wks gestation with IUGR, a severe ventricular disproportion with a hypoplastic left ventricle. Cultured amniocytes revealed a 45,X,der(X)t(X;13);(q28;q12.1),-13 karyotype. The child was born at 40 wks gestation. Her height was 43cm, weight 2190g, and was found to have multiple dysmorphic features and congenital anomalies including turricephaly, prominent forehead, hypertelorism, downslanting palpebral fissures, broad flat nasal bridge, dysplastic ears, long thumbs and long big toes. Postnatal cytogenetics studies revealed that there was skewed X inactivation, with the normal X chromosome inactivated in 83.4% and the der(X) inactivated in 16.6% of the metaphases observed. FISH using a (TTAGGG)<sub>n</sub> probe demonstrated an interstitial telomere on the der(X) chromosome, signifying that the breakpoint was not at the terminal end of Xq28 but at qter. Cardiac exam confirmed a small, but not hypoplastic, left ventricle, a dysplastic pulmonary valve, a large patent ductus arteriosus and pulmonary hypertension. Brain CT scan revealed a partial corpus callosum agenesis. She was surgically treated for gastroesophageal reflux and a hiatal hernia at 4 mths old and was enucleated for unilateral retinoblastoma at 12 mths old. The occurrence of balanced/unbalanced structural anomalies involving X autosome translocations is very rare. Inactivation and spreading effect contribute to the severity of the phenotype and pathogenesis. This patient displays clinical manifestations of functionally monosomic 13q owing to the spreading of X inactivation.

**Y-chromosome specific sequences detection by PCR and FISH analysis in genomic- and gonadal-DNA in patients with Ullrich-Turner syndrome.** *F.J. Alvarez Nava, M. Soto-Alvarez, L. Borjas-Fajardo, M.A. Sanchez, A. Rojas-Atencio, E. Fernandez, M.C. Martinez-Basalo.* Unidad de Genetica Medica, Universidad del Zulia, Maracaibo, Zulia, Venezuela.

Ullrich-Turner syndrome (UTS) is associated in 40-60% with 45,X karyotypes, whereas the remaining cytogenetics findings have structural anomalies in second sexual chromosome (X or Y) in form pure or with mosaicism. It has been proposed that all females with UTS and 45,X karyotypes carry a cell line containing two sex chromosome at low level of mosaicism. This mosaicism is undetectable by standard cytogenetics analysis. Theoretically, this hidden mosaicism may has a chromosome Y in 40% of the cases. Gonadal dysgenesis seen in UTS patients is associated with gonadoblastoma when Y-chromosome derived material is present in their genome. So it is crucial to carry out molecular analysis in these subjects to rule out Y-sequences. The present study was performed to examine the presence of Y-chromosome material in DNA from lymphocytes and gonads in UTS patients. Fifty Venezuelan patients were screened by polymerase chain reaction (PCR) to detect Y-chromosome-derived fragments. Patients with virilization and/or with positive Y-sequences by PCR underwent gonadectomy. Three patients were positive in blood peripheral-DNA to Y-sequences specific. None exhibited signs of virilization. One of them presented gonadoblastoma and dysgerminoma contralateral at 4 years old. She had received treatment with growth hormone. All patients had Y chromosome material in gonads. FISH confirmed their Y-chromosome origin. Our results suggest that the molecular analysis must be applied for detection of Y-chromosome in UTS patients before onset of therapy with HG.

**Duplication of 8p23.1: Characterization of 8 new cases.** *S.L. Graw*<sup>1</sup>, *C.-H. Tsai*<sup>2</sup>, *L. McGavran*<sup>3</sup>. 1) Eleanor Roosevelt Inst, Denver, CO; 2) Department of Pediatrics, Division of Genetics and Metabolism, The Children's Hospital, Denver, CO; 3) Colorado Genetics Laboratory, Department of Pathology, University of Colorado Health Sciences Center, Denver, CO.

Multiple patients with rearrangements involving 8p23.1 have been reported, including inverted and tandem duplications of 8p23.1, deletions of 8p23, and pericentric inversions (p23q22). Recently, Barber et al. (1998) reported 7 new cases of duplication of 8p23.1, and reviewed 5 previously reported cases. O'Malley and Storto (1999) reported another case ascertained prenatally with isolated duplication of 8p23.1. Both studies concluded that this duplication has no clinically identifiable phenotype, and represents an inconsequential anomaly. The vast majority of these cases were ascertained through prenatal diagnosis, and in many cases there has been no long-term follow-up to examine the possibility of developmental delay or other problems that may be revealed after the neonatal period. Here we report 8 new families with duplication of 8p23.1 ascertained at a tertiary care facility with both outpatient and inpatient genetic services. Our data suggest that cardiac abnormalities, short stature, hypotonia, and mild developmental delay are preferentially associated with this chromosomal anomaly. In some cases, the cardiac defects were significant, and resulted in the deaths of 2 patients in the neonatal period. In addition, we identified a preponderance of male (9 of 13 total) carriers of duplication of 8p23.1. Other findings in individual patients with duplication of 8p23.1 include autistic-like features (found in 2 patients), seizures, scoliosis, ulnar stenosis, and mild organomegaly. Additional molecular and cytogenetic analyses are underway to determine the molecular basis behind the dup8p23.1 chromosome formation.

**Sex reversal 2001: Some answers, more questions.** *J.J. Dowhanick-Morrisette<sup>1</sup>, L. Celle<sup>1</sup>, C. Robert<sup>2</sup>, L. Bason<sup>1</sup>, D. McDonald-McGinn<sup>1</sup>, D.A. Driscoll<sup>2</sup>, E.H. Zackai<sup>1</sup>, N.B. Spinner<sup>1</sup>.* 1) Dept Human Gen & Molec Biol, Children's Hosp Philadelphia, Philadelphia, PA; 2) Department of Genetics, University of Pennsylvania, Philadelphia, PA 19104.

Sex is determined by a regulatory cascade of sex-linked and autosomal genes, with approximately 1/20,000 individuals demonstrating abnormalities of sex determination. The study of sex reversed individuals (XX males and XY females) has played a large role in the elucidation of the genes involved in these pathways. Many of the genes involved in sex determination encode transcription factors such as SRY, SOX9, DAX-1, Wnt-4 and SF1, which are expressed during gonadal development. XY females have been found to have mutation or loss of the SRY gene, the androgen receptor gene (AR), duplication of DAX-1, and several malformation syndromes. The majority of XX males have been found to have SRY translocated to an autosome, and rarely duplication of SOX9. There is no explanation for the sex reversal in 20-30% of XX males. We report 5 cases of sex reversal, two 46,XY females and three 46,XX males. Case 1 is an infant who presented with bilateral inguinal hernias and undescended testicles were identified. Family history was significant for a maternal aunt, known to be a 46,XY female. Chromosome analysis demonstrated a 46,XY,inv(X)(q11.2q28) karyotype. FISH using the AR gene demonstrated a split signal on each side of the inversion breakpoint, consistent with disruption of the AR. Case 2 is a XY female who presented at 14 years of age with short stature and mental retardation and molecular studies are pending. Cases 3 and 4 are both 46, XX males who were studied because of dysmorphic features, with normal male genitalia. PCR and FISH for SRY were done and found to be negative in both patients. Additional studies included analysis of SOX9 and Wnt-4, which were both normal by FISH analysis. Case 5 is an XX male who was found to have a submicroscopic rearrangement involving the SRY gene, with translocation to one X chromosome that was identified by FISH. These cases illustrate the wide range of explanations for sex reversal. Further studies are in progress to explain the sex reversal in patients 2,3 and 4.

**Molecular cytogenetic and phenotypic characterization of a four generation family with partial trisomy 9p. S.**

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Since the first case of partial or complete trisomy 9 was described in 1970, thousands of cases has been described making chromosome 9 anomalies one of the most frequent. In most of the described cases in medical literature, the trisomic segment was inherited from reciprocal translocation and to a lesser extend due to di-novo duplications. We will present a four generation family with 11 affected individuals with partial trisomy 9p and 10 asymptomatic balanced translocation carriers resulting from a familial malsegregation of an insertional translocation involving chromosomes 9 and 10. Characterization of the 9;10 translocation was performed by using Multicolor-FISH technology and confirmed with spectrum orange and green painting probes. Examination of the G-banding pattern suggested an inverted insertion of a segment of 9p into band q25.2 of chromosome 10. 82% of the non affected family members are carriers of the balanced translocation suggesting a preferential meiosis drive. No reproductive problems or miscarriages were reported in any of the balanced translocation carriers. Dysmorphic features of affected individuals include high palate, protruded and large ears, flat occiput, clinodactyly of fifth digits, small penis and testicular volume. Short as well as tall stature was observed in individuals with the partial trisomy. To our knowledge this is the first instance of partial trisomy 9p occurring in four consecutive generations.



**Double partial trisomy 15q;17q, in a boy by a 3:1 segregation of a balanced translocation.** *G. Castineyra<sup>1</sup>, J. Herrera<sup>1</sup>, M.E. Mollica<sup>2</sup>*. 1) Genetics, Lomas de Zamora, Fundacion Cientifica del Sur, Buenos Aires, Argentina; 2) Centro Nacional de Genetica Mdica, Buenos Aires, Argentina.

We report a case of 13 years old follow up since he was born. Parents are not consanguinious and had previously a normal girl and two pregnancy lost. He present severe mental retardation, psycologic impairment, self mutilation, short stature and phenotype abnormalities. from cytogenetics studies using G banding techniques and confirmed by Fish, he present an extra chromosome derivated 15. His karyotipe is 47,XY,der(15)t(15;17)(q11.2,q23). This unbalanced finding was derived from a balanced translocation (15q;17q) in the normal father, also present in his daughter. Main clinical features in the proband included those typical findings in the described distal trisomy 17q; but also some of the proximal trisomy 15q liike: moderate hearing loss, lack of speech but presence of others reasonable madurative patterns and anterior displaced anus. He doesn't present convulsive disorders and congenital heart disease. By imging techniques other anomalies were found. This patient is compared with other reported cases in the literature. We suggest that described different phenotypes are due to the chromosomes involved in the rearregment, making counseling difficult.

**Ascertainment of chromosome 22q11.2 microdeletion in congenital heart defects.** *M.G. Bialer<sup>1</sup>, A. Yenamandra<sup>2</sup>, R. Klein<sup>1</sup>, P. Koduru<sup>2</sup>, L. Mehta<sup>1</sup>.* 1) Dept Pediatrics, Div Medical Genetics; 2) Cell Genetics Lab/Pathology, North Shore Univ. Hospital/NYU Medical Center, Manhasset, NY.

Microdeletion of chromosome 22q11.2 is associated with a highly variable phenotype that includes diGeorge, velocardiofacial and Opitz syndromes as well as apparently isolated congenital heart defects and cleft palate. Results of testing at our center since 1996 are summarized. Of 106 patients tested with the diGeorge syndrome critical region probe, Oncor/TUPLE1(Vysis), 12 were deleted (11%). Information was reviewed on these 12 pts. and 68 non-deleted pts. The commonest reason for referral was congenital heart defect (CHD)(60/80). Other indications included multiple congenital anomalies or cleft palate. Most pts. were neonates (57/80). Heart defects in deleted pts. were tetralogy of Fallot (TOF)(8/12), interrupted aortic arch (IAA)(3/12)and IAA with truncus arteriosus (TA)(1/12). Overall 9/17 (50%) pts. with TOF were deleted and 4/5 (80%) pts. with IAA. Deletions were not identified in 9 pts. with transposition of great arteries, 1 pt. with TA, 3 pts. with hypoplastic left heart syndrome or pts. with multiple congenital anomalies with or without CHD. In neonates, ear and palpebral fissure anomalies and umbilical hernias were present more frequently in deleted pts. Immune deficiency was present in 6/11 deleted pts. Renal, limb, or anal anomalies and significant hypocalcemia were not documented in any deleted pt. One 7 y.o child had severe velopharyngeal incompetence. Six of 24 pts. of Hispanic ethnicity were deleted (25%) compared to 3 of 36 (8.5%) Caucasian European pts. This unexpectedly high incidence in Hispanic pts. may reflect referral bias. There were no familial cases and compliance with requests for parental testing was poor. These results confirm the value of 22q microdeletion testing in CHD, particularly TOF and IAA. Minor dysmorphisms may be clues to this diagnosis but are generally non-specific.

**Tetrasomy 15q25.3-qter Resulting from an Analphoid Supernumerary Marker Chromosome in a Patient with Multiple Anomalies and Bilateral Wilms' Tumors.** *J. Hu*<sup>1,2</sup>, *E. McPherson*<sup>1,2,3</sup>, *U. Surti*<sup>1,2,4</sup>, *S. Hasegawa*<sup>4</sup>, *S. Gunawardena*<sup>3</sup>, *S.M. Gollin*<sup>1,2</sup>. 1) Pittsburgh Cytogenetics Laboratory, University of Pittsburgh Center for Human Genetics, UPMC Magee-Womens Hospital; 2) Dept. of Human Genetics, University of Pittsburgh Graduate School of Public Health; 3) Dept. of Pediatrics, University of Pittsburgh School of Medicine; 4) Dept. of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

A 4 year-old girl developed bilateral Wilms' tumors. She had been followed since birth for apparent Shprintzen-Goldberg Syndrome with macrosomia, long fingers and toes, and craniosynostosis. Cytogenetic analysis of her peripheral blood revealed a de novo supernumerary marker chromosome. This stable marker chromosome is present in 19 of 20 lymphocytes analyzed, as well as in all 40 tumor cells studied. Classical and molecular cytogenetic studies indicate that the marker is derived from an inverted duplication of chromosome 15q25.3-qter and contains a neocentromere. The presence of this marker chromosome in our patient results in tetrasomy 15q25.3-qter. The relationship between her genotype and phenotype are discussed in light of genes, including *IGF1R* and *FES*, mapped to the aneusomic segment.

**Clinical manifestations of Coffin-Lowry syndrome associated with de novo 8p23 duplication.** *T. Kondoh<sup>1</sup>, J. Takano<sup>2</sup>, H. Sugawara<sup>2</sup>, T. Ida<sup>3</sup>, N. Harada<sup>3</sup>, T. Matsumoto<sup>4</sup>, N. Matsumoto<sup>2</sup>, N. Niikawa<sup>2</sup>.* 1) Dept Pediatrics, Nagasaki Univ Sch Medicine, Nagasaki, Japan; 2) Departments of Human Genetics, Nagasaki University School of Medicine, Nagasaki, Japan; 3) Kyusyu Medical Science Nagasaki Laboratory, Nagasaki, Japan; 4) School of Allied Sciences, Nagasaki University, Nagasaki, Japan.

Coffin-Lowry syndrome (CLS) is an X-linked disorder characterized by characteristic, coarse facial appearance, skeletal anomalies, short stature, and mental retardation. Mutations in the CLS gene, *RSK2*, have been identified in a subset of CLS patients. We reported a 10-year-old Japanese boy who had clinical manifestations of CLS and an abnormal chromosome 8. Clinical manifestations included dolicocephaly, large and malformed ears, hypertelorism, antimongoloid slant of palpebral fissures, anteverted nostrils, long philtrum, thick lips, open mouth, long chin, high-arched palate, funnel chest, tapering fingers, hypotonia, short stature, and moderate mental retardation. Mutational analysis on exons 2-22 of *RSK2* in the patient revealed no nucleotide change. FISH studies using probes that are mapped at 8p23 demonstrated a tandem duplication of a segment encompassing ~2 Mb on the abnormal chromosome 8. These findings suggest that the 8p23 duplicated segment may contribute to CLS or CLS-like manifestations. Alternatively, a chromosomal imbalance effect due to the 8p23 duplication may in part contribute to the CLS manifestations.

**Trisomy 18q due to de novo unbalanced translocation, 46, XX,+der(4;18)(q35;q12) in one of the triplets, an outcome of IVF A case report and brief review.** *D.S. KRISHNA MURTHY, K.K. NAGUIB, S.J. ABUL HASSAN, L. BASTAKI, S.A. AL-AWADI.* CYTOGENETICS LABORATORY, MEDICAL GENETICS CENTER, SABAH AREA, KUWAIT.

In vitro fertilization and ICSI has become a widely accepted procedure to assist reproduction in couples with infertility problems. However, I V F, ICSI and other micro-assisted fertilization techniques have also lead to a lively debate and concern on possible associated genetics risks in the newborn. Fewer than 10% of the embryos that are transferred to the uterus after IVF?ICSI?ART implant successfully. Chromosomal abnormalities is one of the major causes for failure to implant and develop, or multiple congenital anomalies in the newborn. About 40% of oocytes recovered in stimulated cycles (super ovulation) have abnormal chromosomal complements (aneuploidy/structural). We report here a female neonate, product of a triplet (1 female and 2 males) outcome of a IVF after 5 years of infertility in the couple. On examination at birth revealed, multiple congenital anomalies including odd facial features, prominent occiput, overriding of skull bones, flat nasal bridge, micrognathia, high arched palate, blue sclera, low set ears, dental cyst, limb deformities (rocker bottom feet) and mild hirsutism, No CHD or organomegaly was noted. The other two male sibs were normal. RE-evaluation at 5 and 18months revealed some clinical features of trisomy 18q and the child is progressing well with moderate developmental delay, no major cardiac or neurological problems. Chromosomal analysis of peripheral blood in the index case showed an abnormal chromosome 4, [46,XX,+der(4),t(4;18)(q35;q12), resulting partial trisomy for 18q. Karyotype of the parents and other two sibs were normal. FISH analysis using WCP for chromosomes 4 and 18 and quint18 essential probe (18q21.2) confirmed 18q trisomy [46,XX,ish(4;18)(q;q) (WCP4++,WCP18+++,18q21.2+++)]. The clinical and karyotypic findings in trisomy 18q cases will be briefly reviewed with regard to the survival and pathogenesis. The significance of chromosomal abnormalities and genetic counselling of the families followed by IVF procedure will be briefly reviewed.

**Holoprosencephaly in sibs with a Robertsonian (14q;22q) translocation chromosome.** *D. Kamnasaran<sup>1</sup>, J.A. Gerritsen<sup>2</sup>, D.R. McLeod<sup>2</sup>, D.W. Cox<sup>1</sup>*. 1) Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada; 2) Department of Medical Genetics, Alberta Children's Hospital, Calgary, Alberta, Canada.

We report the clinical, cytogenetic and molecular findings on two sibs with clinical signs within the broad holoprosencephaly spectrum. The proband had microcephaly, hypotelorism, cleft palate and premaxillary agenesis. After her sudden death at 8 months, an autopsy examination showed partial agenesis of the corpus callosum with an associated malformed cingulate gyri and white matter heterotopia. The other affected sib had microcephaly, brachycephaly, bilateral ptosis, strabismus, high arched palate and a prominent nasal bridge. His cranial CT scans were normal. Standard G banding karyotype analyses were performed on the chromosomes of family members using cultured peripheral blood lymphocytes and/or prenatal amniocytes. Both affected sibs were found to be carriers of a familial robertsonian translocation (14q;22q)(q10;q10) chromosome inherited from the maternal lineage. Since robertsonian translocations are common in the genesis of uniparental disomy (UPD), genotyping was performed with a panel of 32 chromosomes 14 and 22 polymorphic markers in search of uniparental disomy. DNA sample was unavailable from the proband. We found no evidence of partial or complete maternal or paternal UPD 14 or UPD 22 in the affected brother, based on 27 informative genotypes of markers mapping to chromosomes 14 and 22. The abnormal phenotypes observed in the affected sibs also suggested the presence of an additional rearrangement of the familial robertsonian t(14q;22q) chromosome. We searched for microdeletions in the affected brother in the 14q13 region, a candidate holoprosencephaly locus. We found no microdeletion based on 18 informative markers mapping between D14S297 and D14S1013, by PCR analysis of flow sorted t(14q;22q) chromosomes. The phenotype of the affected brother is not apparently due to UPD 14 or 22, or to a detectable microdeletion. The phenotype maybe due to a recessive gene inherited from both parents or dominant gene with variable expression inherited from a parent with incomplete penetrance. The possibility of an additional rearrangement in the proband cannot be excluded.

**Subtle subtelomeric deletion in 8p23.3 in a child with developmental delay and dysmorphism detected by FISH: Paternal origin and molecular confirmation.** *X.L. Huang, A. Pan, H.E. Wyandt, J.M. Milunsky.* Center for Human Genetics, Boston Univ Sch Medicine, Boston, MA.

A 1-year-old female had dysmorphic features and developmental delay suggestive of Down syndrome. Height, weight, and OFC were in the 75th centile. She had a flat face, protruding tongue, bilateral epicanthal folds, brushfield spots, a broad short nose with anteverted nares, a left simian crease, 5th-finger clinodactyly, and hypotonia. Mother and child had similar up-slanting palpebral fissures. Chromosomes of the child and her parents were normal by high resolution banding. Subtelomeric FISH revealed a weak or absent signal on a chromosome 8 (8p23.3), compared to its homolog, with the probe D8S504. Signal size-discrepancy was also noted in interphase cells, many having only one signal. D8S504 gave equal-size signals in chromosomes 8 from the mother. Signal size in chromosomes 8 from the phenotypically normal father was discrepant, but no cells were missing a signal. A probe for a more distal locus in 8p23.3, PAC580L5, showed no size-discrepancy by FISH in any of the family. Eight microsatellite markers in the subtelomeric region of 8p23.3, NIBg, WI-6641, WI-4250, D8S504, WI-5411, WI 1986, AFMB3222H9 and D8S201 (listed by Gene Bank order) were compared by PCR analysis in the child, her parents and normal controls. The first six markers were informative. The paternal allele for D8S504 is absent in the child, confirming the deletion in 8p23.3. No deletion was evident in the father. Thus, diminished signal size in the father by FISH may represent a normal variant that predisposed to a different size deletion in the child by unequal crossing-over in paternal meiosis. At 16 3/4 months of age our patient has behavioral problems in addition to her clinical findings, including head-banging, self-biting and pinching, frequent rocking behavior, tantrums and tactile defensiveness. The subtelomeric region of 8p23.3 appears to be a hotspot for chromosome rearrangement, with numerous reports of duplications and/or deletions involving breakpoints in this region. Many of the features in our patient are consistent with those reported for 8pter-microdeletions.

**Chromosomal abnormalities in patients with idiopathic hypogonadotropic hypogonadism.** *L.C. Layman<sup>1</sup>, D.P. Bick<sup>2</sup>, R.J. Sherins<sup>2</sup>, A. Kulharya<sup>3</sup>*. 1) Section of Reproductive Endocrinology, Infertility, & Genetics, Dept OB/GYN, Neurobiology Program, Institute of Molecular Medicine & Genetics, Med Col GA, Augusta, GA; 2) Genetics & IVF Institute, Fairfax, VA; 3) Cytogenetics, Dept Pediatrics, Med Col GA, Augusta, GA.

Humans with hypogonadism are generally categorized into hypergonadotropic (gonadal failure) or hypogonadotropic (hypothalamic/pituitary deficiency) hypogonadism. The probability of a chromosomal abnormality is markedly increased in patients with hypergonadotropic hypogonadism, with more than 50% of females with primary amenorrhea and 10-15% of males having an abnormal karyotype. It is generally thought that idiopathic hypogonadotropic hypogonadism (IHH) patients, except for those with Prader-Willi syndrome, do not have chromosomal abnormalities, although there are no studies of well-documented IHH patients. Identified karyotypic abnormalities would not only document the prevalence of chromosomal disorders, they might also provide clues to genes involved in the pathogenesis of IHH. The molecular basis for most patients with IHH remains unknown, although mutations in the KAL1, AHC, GNRHR, LEP and LEPR genes have been identified in a minority. We undertook a study to determine the prevalence of karyotypic abnormalities in humans with IHH. Peripheral blood white blood cells or lymphoblastoid cell lines were collected from 50 well-documented IHH patients and a metaphase karyotype, counting 20 cells, was performed. IHH was defined as absent puberty (at least age 17 in females and 18 in males), low serum levels of FSH and LH, normal levels of other pituitary hormones, and the absence of a pituitary tumor. One male had a 46,XY,t(10;12)(q26.3;q13.1) and another was 46,XY with an abnormal Y in half of cells, which is currently being characterized. The 10q26 breakpoint was involved in a previously described IHH patient, suggesting the relevance of this chromosomal region in IHH. Our findings suggest that chromosomal abnormalities may be more common (4%) than previously expected, and that these aberrations may provide clues to additional genes involved in the pathogenesis of IHH. (L.C.L. supported by PHS-NICHD HD33004).



**Pelizaeus-Merzbacher disease with unusual presentations of phrenic nerve paralysis and AV block.** *J. Lin<sup>1</sup>, S. Chang<sup>2</sup>, S. Huang<sup>3</sup>.* 1) Genetic Div Pediatric Dept, Chang Gung Children's Hosp, Taoyuan, Taiwan; 2) Dept. of GYN & OBS, Chang Gung Memorial Hospital, Taoyuan, Taiwan; 3) Dept. of Pathology, Chang Gung Memorial Hospital, Taoyuan, Taiwan.

Pelizaeus-Merzbacher disease was an dysmyelinating disorder, presented with diffuse cerebral sclerosis and projectile vomiting etc. A male newborn with IUGR was intubated after birth due to low Apgar score. Dysmorphism was noted after birth. Phrenic nerve paralysis were found later. He developed second degree AV block Mobitz type II around one month old. Temporary pacemaker was inserted. Autopsy was done after he died of septic shock at five months old. Myelination defect was found by electromicroscopy. Blood karyotyping of patient showed 46, XY, dup(X)(q21-q22). The karyotyping of mother showed 46, XX, dup(X)(q21-q22). The mother was physically normal without any dysmorphic picture noted. This is the first case of Pelizaeus-Merzbacher disease ever reported at Taiwan, with unusual presentation after birth. Blood karyotyping and nerve histopathology by electromicroscopy will be presented and discussed.

**Deletion of the 3q27-qter region associated with a congenital dyserythropoietic anemia.** *C. Michel<sup>1</sup>, V. Drouin-Garraud<sup>2</sup>, P. Schneider<sup>1</sup>, C. Bastard<sup>3</sup>, J.P. Vannier<sup>1</sup>, T. Frebourg<sup>2</sup>.* 1) hemato-cancerologie , CHU de Rouen, Rouen; 2) Service de genetique, CHU de Rouen; 3) Centre Henri Becquerel, Rouen.

The deletion of the 3q27-qter region has been described in few patients with multiple congenital abnormalities. We report the case of a 17 year-old girl with a de novo deletion of the 3q27-qter region, who presented with mental retardation, growth delay, dysmorphic features and a congenital dyserythropoietic anemia (CDA) associated to neutropenia, humoral immunodeficiency and thrombocytosis. This patient had no malformation. This is, to our knowledge, the first case of a 3q27-qter deletion associated to CDA. Three principal types of CDA have been described so far, CDAI (autosomal recessive), CDAIL (autosomal recessive) and CDAILI (autosomal dominant), and the corresponding genes have been mapped to 15q15.1-q15.3, 20q11.2 , and 15q21, respectively. Genetic heterogeneity has been demonstrated for type II. This reports suggests the location of an additional CDA locus on chromosome 3q27.

**Mosaicism for 45,X and a Cell Line with a Derivative Y Chromosome containing 2 Copies of the SRY gene resulting in a Male Phenotype.** A.A. Khan<sup>1</sup>, D. Chitayat<sup>1</sup>, M. Speevak<sup>2</sup>, R. Murphy<sup>3</sup>. 1) Division of Clinical Genetics, Hospital for Sick Children Toronto, Ontario, Canada; 2) Department of Laboratory Medicine, Credit Valley Hospital, Mississauga, Ontario, Canada; 3) Dufferine-Caledon Healthcare, Orangeville, Ontario, Canada.

Mosaicism for 45,X and a cell line containing a Y chromosome has been reported to be associated with features ranging from a male phenotype with gonadal dysgenesis to a female phenotype with normal female genitalia. We report a newborn infant with sex chromosome mosaicism, a male phenotype with complete absence of the Mullerian structures and no stigmata of Turner syndrome. The patient was born at 33 weeks gestation following a pregnancy complicated with poor fetal growth, oligohydramnios and maternal preeclampsia. Delivery was induced and the newborn's birth weight was  $< -2$  SD. On examination he had ambiguous genitalia with grade III hypospadias, chordee and penile length of 2.5 cm and a smooth scrotum. The left testis was descended and the right hemi-scrotum was hypoplastic with undescended testis and hydrocele. The anus was anteriorly placed. No Mullerian structures were noted on pelvic ultrasound and no other abnormalities were detected. Chromosome analysis done on peripheral blood revealed sex chromosome mosaicism: 45,X[99]/46,X,der(Y).ish idic(Y)(q11.2) (wcpY+,SRY++)[8]. In the second cell line, FISH revealed a dicentric Y chromosome with duplication of SRY and absence of Yq heterochromatin. Only a single active centromere was noted. Yq deletion analysis by PCR revealed the presence of the AZF loci. This analysis suggested duplication of Yp and deletion of a part of Yq in 7.5% of cells in the peripheral blood. The possible origin of this complex chromosome abnormality and the genotype/phenotype correlation will be discussed.

**Cloacal extrophy in an infant with 9q34-qter deletion resulting from a de novo unbalanced translocation between chromosome 9q and Yq.** *C. Thauvin-Robinet<sup>1</sup>, P. Khau Van Kien<sup>1</sup>, M. Fellous<sup>2</sup>, K.L. Parker<sup>3</sup>, D. Semama<sup>4</sup>, I. Luquet<sup>5</sup>, I. Sidaner-Noisette<sup>5</sup>, A. Nivelon-Chevallier<sup>1</sup>, F. Mugneret<sup>5</sup>, L. Faivre<sup>1</sup>.* 1) Centre de Genetique, Hopital d'Enfants, Dijon, France; 2) Service d'Immunogenetique Humaine, Institut Pasteur, Paris, France; 3) Department of Medicine, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina, USA; 4) Service de Pediatrie 2, Hopital d'Enfants, Dijon, France; 5) Laboratoire de Cytogenetique. Hopital Le Bocage, Dijon, France.

Cloacal extrophy is a rare malformation, which occurs in 1:200,000 to 1:250,000 live births. It belongs to a spectrum of birth defects, which, in order of severity, includes phallic separation with epispadias, pubic diastasis, bladder extrophy, cloacal extrophy and OEIS complex (omphalocele, extrophy of bladder, imperforate anus, and spinal defects). The pathogenesis of cloacal extrophy is unknown to date. It may result from a single defect of early blastogenesis or a defect of mesodermal migration during the primitive streak period. Here, we report on an infant with cloacal extrophy, rectal prolaps, absence of bladder, right renal agenesis, sexual ambiguity and axial hypotonia. Chromosome analysis showed a de novo unbalanced translocation between the long arm of chromosome 9 and the long arm of chromosome Y resulting in a 9q34-qter deletion (46,XY,der(9),t(Y;9)(q34;q12)). Reviewing the literature, we did not find cloacal extrophy associated with structural chromosomal abnormality. We sequenced the gene SF1 (Steroidogenic Factor 1) included in the deleted region but no mutation was found. We hypothesize the localization of a gene, early expressed in embryogenesis and responsible of cloacal extrophy, in the 9q34-qter region.

**An additional patient with del(12)(q21.2q22): Further evidence for a candidate region for Cardio-facio-cutaneous syndrome?** *K. Rauen<sup>1</sup>, D. Albertson<sup>2</sup>, P. Cotter<sup>1,3</sup>*. 1) Division of Medical Genetics, Department of Pediatrics, University of California, San Francisco, CA 94143; 2) Cancer Research Institute, University of California, San Francisco, CA 94115; 3) Division of Medical Genetics, Childrens Hospital Oakland, 747 Fifty Second Street, Oakland, CA 94609.

Cardio-facio-cutaneous (CFC) syndrome is a rare MCA disorder in which individuals have a characteristic facial appearance, cardiac defects, ectodermal anomalies and developmental delay. Recently, we reported a girl with the phenotype of CFC who had an interstitial deletion of the long arm of chromosome 12, del(12)(q21.2q22), implicating a possible locus for CFC syndrome (AJMG.2000.93:219-222). Since that report, we have an additional patient who carries the same deletion. Standard cytogenetic analysis shows a 47,XYY karyotype containing an interstitial deletion of the long arm of one chromosome 12 observed in all metaphases examined: 47,XYY,del(12)(q21.2q22). Examination of this patient by the use of photographs reveals craniofacial features including a broad forehead, hypoplasia of the supraorbital ridges, mild down-slanting palpebral fissures, short nose with bulbous tip and low-set auricles. Ectodermal findings consist of sparse, curly hair (which was more pronounced during infancy), no eyebrows, scant eyelashes and hyperkeratosis pilaris. Prenatal history is significant for polyhydramnios, a 2-vessel cord and right hydronephrosis on ultrasound. Medical history includes a small PDA and persistent patent foramen ovale closing after several months of age, pyloric stenosis at age 3 weeks, right hydronephrosis and right cryptorchidism. The patient is delayed developmentally and has a normal brain MRI. To investigate CFC patients for submicroscopic chromosome aberrations, we have employed the use of comparative genomic hybridization microarrays (CGHa) to rapidly and efficiently screen the genome for submicroscopic deletions in the candidate region. CGHa analysis of our male patient confirmed the cytogenetically visible deletion in one chromosome 12, as well as detected the 2 copies of the Y chromosome. Current studies are underway to examine additional patients who have been clinically diagnosed with CFC using CGHa.

**High-throughput analysis of subtelomeric rearrangements using array-based CGH.** *J.A. Veltman<sup>1</sup>, A.J.A. van Kampen<sup>1</sup>, B.H. Eussen<sup>2</sup>, E.F.P.M. Schoenmakers<sup>1</sup>, C.M. van Ravenswaay<sup>1</sup>, A. de Klein<sup>2</sup>, D.F. Smeets<sup>1</sup>, H.G. Brunner<sup>1</sup>, A. Geurts van Kessel<sup>1</sup>.* 1) Human Genetics, UMC Nijmegen, Nijmegen, Netherlands; 2) Clinical Genetics, Academic Hospital Rotterdam, Rotterdam, Netherlands.

Telomeric rearrangements result in a number of clinical conditions, including mental retardation, congenital anomalies and recurrent miscarriages. Automated detection of subtle deletions or translocations involving telomeres is essential for high-throughput diagnosis but impossible with the conventional cytogenetic methods. Array-based comparative genomic hybridization (CGH) has been developed for high-resolution screening of copy number abnormalities by hybridizing differentially labeled test and reference genomes to arrays of robotically spotted clones. In this double-blinded study DNA from 40 patients with known cytogenetic abnormalities involving one or more telomeres was hybridized to an array containing a second-generation set of human chromosome-specific (sub)telomere probes. Single copy number gains and losses were accurately detected on these arrays and there was an excellent concurrence between the original cytogenetic diagnosis and the arrayCGH-based diagnosis using a single hybridization. We have confirmed the suitability of this method by analyzing DNA from a large set of patients with nonspecific mental retardation for submicroscopic rearrangements. The robustness and simplicity of this array-based telomere screening makes it highly suited for introduction into the clinic as an automated diagnostic procedure.

**Digital, spinal and other anomalies in a patient with a whole arm chromosome translocation resulting in monosomy 18p and trisomy 20p.** *K.L. Russell, N.B. Spinner, J.E. Ming.* Div Human Gen & Molec Biol, Children's Hosp Philadelphia, Philadelphia, PA.

Non-Robertsonian whole arm chromosomal translocations are rare events. We present the third case of a whole arm translocation resulting in monosomy 18p and trisomy 20p. The patient is a five month old girl born at 41 weeks gestation to a 30 year old G6P2 mother after an uncomplicated pregnancy. Length, weight and head circumference at birth and five months were normal. On the left hand, there was a duplicated index finger with two phalanges, and there was cutaneous syndactyly between the two index fingers and the thumb. The right hand had a sharply angled ulnar deviation of the second digit at the proximal interphalangeal joint. The patient also displayed brachycephaly with plagiocephaly, blepharophimosis, ptosis, low set ears, a broad nasal root, down-turned corners of the mouth, micrognathia, irregular toe placement and an umbilical hernia. Spinal anomalies included cervical spine fusion of C5-C7, thoracic lordosis and lumbar kyphosis. She also had a deep sacral dimple, developmental delay and bilateral sensorineural hearing loss. Her karyotype was 46,XX, der (18),t(18;20)(p11.1;q11.1). The centromere of the derivative chromosome contained alpha satellite sequences from both chromosomes 18 and 20. The derivative chromosomes in the two previously reported cases also had centromeric material from both chromosomes. This patient's clinical findings are similar to the two other reports of monosomy 18p/trisomy 20p. The patient presented here had more severe hand malformations than the previously reported cases and additional findings of spinal fusion and hearing loss. This case further demonstrates that 1) for this chromosomal rearrangement, the resulting derivative chromosome has a break in the centromere, possibly indicating that there are homologous sequences in the alpha satellite regions of chromosomes 18 and 20, and 2) monosomy 18p/trisomy 20p has a definable phenotype that includes brachycephaly with a distinctive facial appearance, finger anomalies, irregular toe placement, umbilical hernia and hypotonia.

**Inherited duplication of Xq26.1q27.2, including SOX3, in mother and daughter with short stature and dyslalie. I.**

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Duplications of the distal long arm of the X chromosome are rare and carrier females are usually phenotypically normal. We report a short statured girl who inherited a dup(Xq26.1q27.2) chromosome from her short mother. At the age of 12 years her height was 131.6 cm (<3rd percentile), weight 30.6 kg (3rd percentile). She had minor dysmorphic features, lordosis and no menarche nor signs of puberty. She has regular, low prepuberal levels of gonadotrophins and steroids, but borderline low IGFI and IGF-Bp3 serum levels. Both the propositus and her 42-year-old mother (height 152 cm, weight 51.8 kg) with a strikingly "old" face and a prominent nose have severe speech problems with stuttering and dyslalia. Menarche was rather late at the age of 15 years. Until now, no signs for irregular cycles or POF are being reported. Both maternal sisters (155 cm, 153 cm) and her mother (150 cm) are also short. Karyotyping revealed an additional band at Xq26-q27 in all metaphases from daughter and mother. With a chromosome X paint both X chromosomes were completely labeled. By FISH, 8 out of 16 BACs were found to be duplicated on that chromosome. The proximal breakpoint maps between BAC RP11-41L14 (DXS7415) and RP11-614F19 (DXS7315) at band q26.1 and the distal breakpoint between BACs RP11-189F12 and RP11-518F7 at band q27.2. The duplicated 10-11 Mb segment include locus DXS102 and SOX3. The same segment is involved in 2 larger familial duplications, both of which were found to be associated with an abnormal phenotype in males, including short stature (Gecz et al. 1999; Hol et al. 2000). Using the androgen-receptor (AR) gene methylation assay we were able to show that despite preferential inactivation of the dup(Xq) a significant portion of lymphocytes in mother and daughter do carry an active X chromosome with the duplication. A dosage effect of genes such as SOX3 appears to be involved in features like short stature in these 3 families.



**Classical phenotype of trisomy 1q42-qter syndrome in a patient with an inv dup del (1)(pter→q44::q44→q42:).** *G. Sebastio<sup>1</sup>, D. De Brasi<sup>1</sup>, E. Rossi<sup>2</sup>, S. Giglio<sup>2</sup>, A. D'Agostino<sup>3</sup>, L. Titomanlio<sup>1</sup>, C. Di Stefano<sup>1</sup>, G. Andria<sup>1</sup>.* 1) Dept Pediatrics, Federico II Univ, Naples, Italy; 2) Dept of Medical Biology, University of Pavia; 3) Dept of Biology and Molecular and Cellular pathology, Federico II University, Naples.

Inverted duplications are cytogenetic rearrangements due to meiotic errors during homologous chromosomes pairing and are always associated with telomeric deletions. We describe a girl with an inv dup del (1)(pter→q44::q44→q42:), who showed macrocephaly with prominent forehead, down-slanting palpebral fissures, micrognathia, mild dilated cardiomyopathy and psychomotor retardation. Standard cytogenetics revealed a duplication of the tract 1q42-q44. Chromosome 1 painting revealed an uniform signal across both chromosomes 1. FISH performed with telomere specific clones demonstrated a terminal deletion of one chromosome 1 within region q44. Double-colour FISH with YACs from 1q demonstrated a duplication of more than 30 cM and an inversion of the duplicated region. Eventually, complete karyotype was: 46,XX, inv dup(1)(q44q42).ish(dup del 1)(q44q42)(D1S446x2, D1S423x2, tel1q-). "Direct" and "inverted" duplications should be accurately differentiated as inverted duplications are invariably associated with telomeric deletions. To distinguish between these two types of rearrangements, FISH analysis is often necessary. The phenotypic effects of these complex rearrangements are only partially known. The location and extension of the deleted segment and the breakpoints of the inversion, as well as the length of the trisomic region, may influence the phenotype. Clinical findings of the present patient mostly overlap that observed in patients affected by the typical trisomy 1q42-qter syndrome. This is probably because of the small extension of the deleted chromosomal segment, with a limited phenotypic effect. Though not included in the minimal clinical criteria, mental retardation, observed in our patient and described with variable degree in all the cases of the trisomy 1q42-qter reported so far, should be considered as a constant feature of the syndrome.

**Ambiguous Genitalia: Clinical, Chromosomal and Ultrasonographic Study..** *N.A. Abukarsh<sup>1</sup>, N.L. Hatem<sup>2</sup>, N.E. El-Kholi<sup>3</sup>, A. Abdel-Kareem<sup>4</sup>, F.M. Kamel<sup>2</sup>, S.M. Tayel<sup>5</sup>.* 1) Histology Department, Faculty of Medicine, Great Al-Fateh University, Tripoli, Libya; 2) Pediatrics Department; 3) Department of Pediatric Surgery; 4) Radiology Department; 5) and Clinical Cytogenetics Unit, Anatomy Department, Faculty of Medicine, Alexandria University, Egypt.

Ambiguous genitalia (AG) in the newborn represents a true medical emergency. It may be accompanied with other serious birth defects and it may lead to family psychosocial crisis. Diagnosis of AG has to be started as early as possible to allow proper sex assignment and to avoid any change in the sex of rearing. Twenty patients with AG (age range: 1 week - 11 years, mean = 3.8 years) were subjected to general and local genital examination including measurement of clitoral breadth and stretched penile length, karyotyping by GTG- banding, pelvic ultrasonography, and hormonal assay. Results showed 13 patients (65%) as male pseudohermaphrodites due to testosterone and 5 $\alpha$ - reductase deficiencies, and testicular feminization syndrome (TFS), 2 (10%) of patients were diagnosed as female pseudohermaphrodites due to congenital adrenal hyperplasia (CAH), and 5 patients (25%) had AG due to nonendocrine/non sex chromosome defects in the form of VATER association, trisomy 13, Russell-Silver, Prader-Willi, and Frazer syndromes. Parental consanguinity was present in 15 cases (75%). Eleven of them (55%) showed autosomal recessive disorders. One of the male pseudohermaphrodites (5 $\alpha$ - reductase deficiency) had 5 sibs affected, 3 of them (aged 20, 21, and 22 years) were wrongly assigned the female sex. Three patients (15%) changed gender after proper sex diagnosis. Gender role has to obey chromosomal sex in certain disorders as CAH, testosterone and 5 $\alpha$ - reductase deficiencies. It goes to the opposite sex in TFS. Phallus size, pattern of pubertal change expected at adolescence and family desire are the factors to be taken into consideration when deciding the more appropriate gender of patients with AG. Genetics work-up is crucial for early and proper sex assignment in case of AG to avoid gender change and to give accurate recurrence risk estimation in other sibs especially in communities with high consanguinous mating as arab countries.

**Low Sex Ratio in Patients With Clinical Diagnosis of Down Syndrome.** *N.V. Kovaleva.* Institute of Obstetrics and Gynaecology RAMS, St.Petersburg, Russia.

Male to female ratio (sex ratio, SR) was studied in 1329 cases of cytogenetically confirmed trisomy 21 (group I) and in 538 clinically diagnosed cases of Down syndrome (group II) born in St. Petersburg in 1970-1999. SR was increased in cases confirmed cytogenetically (731 males and 595 females, SR=1.23), but not in patients who did not receive chromosome analysis (263 males and 275 females, SR=0.96), the difference in SR between groups is significant ( $p=0.0143$ ). Mothers of girls from group II were younger than mothers of girls from group I, the difference between proportion of mothers aged 35 and older (23% versus 33%) is significant,  $p=0.0046$ . Infant mortality was higher in group II (79.5%) than in group I (53.9%), the difference is highly significant ( $p<0.001$ ). Previous reports indicate that mosaic trisomy 21 cases show a SR  $<1.0$ , suggesting that a large proportion of the group II cases represents mosaics. However these results do not support an excess of mosaic trisomy in the group II. Among children referred to geneticists because of having some clinical features resembling Down syndrome but with normal karyotypes in blood (group III), a high female preponderance was found; there were 55 cases of 46,XY males and 141 of 46,XX females (SR=0.39), significantly different from population value 1.06,  $p<0.0001$ . Therefore, low SR in clinically diagnosed cases may be explained by an excess of false-positive diagnosis of Down syndrome in females. Thus, these results would suggest that epidemiological data collected on Down syndrome, prior to routine cytogenetic analysis, should be considered suspect and perhaps discarded. Furthermore, the high proportion of females in group III suggests an etiology specific to females, such as Rett syndrome, X-inactivation anomalies, UPD, or other aberrations of the X chromosome.

**Pericentric inversion of chromosome 12(p13q24) in a family; phenotypic variability and reproductive risk.** *P.D. Power<sup>1</sup>, L.T. Arbour<sup>1</sup>, H. Bruyere<sup>1</sup>, C. Pushpanathan<sup>2</sup>, E.J. Ives<sup>1</sup>.* 1) Medical Genetics, UBC, Vancouver, BC, Canada; 2) Pathology, Memorial University of Newfoundland, St. John's, NF, Canada.

Among previously reported pericentric inversions of chromosome 12 there is only one report with breakpoints at p13 and q24 (Speleman et al. 1993). We report a further large family with these breakpoints in which information is available on five abnormal cases, including an aborted fetus, all of which were female. Four have the same unbalanced karyotype, 46, XX, rec (12) dup (12q) inv (12) (p13q24), and it is presumed in the fifth. Four of the five cases were examined by the authors. Features of affected individuals include a normal head circumference at birth with subsequent microcephaly in some and macrocephaly in others, bitemporal narrowing, frontal bossing, with supraorbital hypoplasia, and generalized post-natal failure to thrive. Septal heart defects were present in 3 cases. An iris coloboma was present in one infant, and bilateral retinal colobomas were present in another. One child died of primary pulmonary hypertension at age 9 months as a result of generalized congenital capillary hemangiomas. Another died at 3 years of age, and the remaining two are alive at ages 9 and 33 years. All affected individuals were cognitively impaired, but the degree varied. The phenotypic presentations in this kindred are dissimilar to that of the previously reported family (Speleman et al. 1993). In our family, the risk for a carrier of a balanced inversion to have an abnormal pregnancy that will proceed to viability is 20%, with a 10% risk for spontaneous abortion. Male carriers are as likely to transmit as females and there appears to be no impact on fertility or early pregnancy loss in this family. This information may be useful for other families with a similar chromosome rearrangement.

**Proteus Syndrome: Analysis of 7 Cases of a Clinical Genetic Service.** *A.X. Acosta, D. Nunes-Silva, C. Fortuna, M.B. Toralles.* Hospital Universitário Professor Edgard Santos Universidade Federal da Bahia, Salvador-Bahia-Brasil Condomínio das Bolandeiras 2, Imbuí-Salvador, Brazil.

Proteus Syndrome (PS) is a rare and highly variable hamatomatous disorder comprising malformations and overgrowth of multiple tissues. It is characterized by a mosaic distribution of lesions, progressive course, sporadic occurrence, hyperplastic lesions of connective tissue, vascular malformations, linear verrucous epidermal nevi, and hyperostoses. The diagnoses of this pathology are difficult because it is so variable on its presentation, and is frequently confused with hemihyperplasia. At the period of 1998 to 2001, the genetic ambulatory from the University Federal Hospital from Bahia, Salvador - Brazil received close to 750 patients. Seven of these patients were remitted to our service because they were questioned to have PS. There were 4 female and 3 male, and the age at the moment of the attendance varied of five months to eleven years old. All of them were examined and we used the diagnostic criteria proposed by Biesecker [Am J Med Genet 84:389-395 (1999)]. In our sample, the most common characteristic was the hemihyperplasia (6 of 7). Five of them were considered affected to PS. The epidermal nevi were present in all patients that were diagnosed with PS (5 of 7), although none of them had connective tissue nevus. Hemangioma was quite common (2 of 5), and lipoma was rare (1 of 5). The diagnosis of this disorder is difficult because PS has a variable presentation that can appear in the evolution of this illness. It is important to use a clinical protocol to make a correct diagnosis for this syndrome; be attempt and follow cases presenting hemihyperplasia. Finally, this syndrome doesn't appear to be so rare, maybe the suspected cases are not being correctly evaluated.

**The serotonin connection: a family history study of the possible association between autism and migraine headaches.** *E.S. Gordon, N.J. Minshew, E. Gettig, B.J. Devlin.* University of Pittsburgh, Pittsburgh, PA.

Objective: Evaluate the odds of experiencing migraine headaches (without aura) in autistic probands and their first degree relatives compared to a control population. Background: Approximately 30% of individuals with autism are known to have increased platelet serotonin levels. Serotonin levels have also been found to be increased in the parents of autistic individuals. Migraine headaches are thought to have a serotonin component due to the successful treatment of migraine headaches with selective serotonin reuptake agonists. Methods: A questionnaire to determine the presence of migraine headaches was developed based on the diagnostic criteria for migraine without aura designed by the International Headache Society. The questionnaire was sent to 224 families (137 families with an autistic proband; 87 families of control subjects) already enrolled in a study of autism at the University of Pittsburgh. Data was entered into a Microsoft Access database, from which a computer algorithm was designed to evaluate the presence or absence of migraine headache in each individual. The statistical package SAS was used to determine any statistically significant differences between the presence of migraine in the family members of autistic probands compared to family members of control subjects. Other headache variables such as location of the headaches, feeling of headache and frequency of headaches were also analyzed. Results: Responses were collected from 65 families (44 families with an autistic proband; 21 control families). A total of 105 responses were collected from parents in these two groups (75 parents of autistic probands; 30 parents of control subjects). No significant differences were found between odds of reporting symptoms meeting diagnostic criteria for migraine in the parents of these two groups. Previous analyses using only 51 families showed no significant differences between the children (siblings and probands combined) in these two groups. Conclusion: There is no evidence to support a common serotonin basis for migraine and autism. Further studies are warranted to investigate the role of serotonin in both autism and migraine.

**Cleft lip and palate anomalies in Patagonia.** *H. Oh, C. Brodsky, J. Mir, T. Mosby, M. Tolarova.* Dpt of orthodontics, UOP School of Dentistry, San Francisco, CA.

**INTRODUCTION.** Cleft lip and palate anomalies represent a major problem in oral health worldwide. Every two minutes, a child affected with orofacial cleft is born somewhere in this world. On average, one out of every 500 newborns has a cleft. As a necessary step towards prevention, epidemiological studies evaluating etiological roles of environmental factors (including nutrition) and of genetic factors are performed.

**MATERIAL AND METHODS.** We studied 72 individuals affected with orofacial cleft and 38 unaffected (control) individuals from the Argentinean Patagonia province Chubut. A diagnostic spectrum, birth weight, birth order, age of parents and month of birth were evaluated.

**RESULTS.** Following results were obtained from an initial analysis of epidemiological characteristics of cases and controls examined in the city of Trelew: The mean age of our cleft patients was 17.8 years. The majority (83%) had cleft lip and palate. In unilateral cases, the left side was affected more often (69%). Children affected with cleft were more likely to be first born or of parity 4 or higher. The mean birth weight was 3209 grams. Evaluation of maternal age revealed a higher prevalence of mothers younger than 24 years for cases, but not for controls. Detailed analyses of epidemiological characteristics were performed in subcategories classified according to cleft severity and laterality. Ongoing analyses include environmental, nutritional and genetic data.

The field work for this study was supported by funding from ROTAPLAST International, Inc.

**Associated factors evaluation in recurrent and/or severe infections in Down's syndrome patients. L.M.A.**

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Down's Syndrome (DS) is the most common cause of mental retardation among the genetic syndromes, with incidence of 1/700 live births, reaching 1/50 live births of mothers with age above 45 years old. Recurrent infections are frequent in DS patients, being considered important cause of morbidity. In this study there were evaluated 45 SD patients with recurrent and/or severe infections, through epidemiological, clinical and laboratorial protocol, including immunological aspects, from jan/90 to jul/99. The sex distribution was 1,6male:1female, with age average=2years 7months (ranging=1y to 12y10mo). Forty patients referred recurrent infections (RI) and five sepsis. Out of all patients with RI, 31 fulfilled the repeated infection criteria, with pneumonia and rhinopharyngitis as the most common infections. The chromosome analysis revealed 41 patients with simple trisomy, 3 mosaicisms and one translocation. Congenital heart diseases were found in 62.5% of cases, more frequent in the repeated pneumonia group. Immunological evaluation showed 2 cases with IgG2 deficiency, 2 with low lymphocytes CD4+ count, 13 cases without response to delayed cutaneous hypersensitivity tests and one of these cases without response to PHA in lymphocytes culture. One patient showed no response to PWM and OKT3 mitogens and 5 with reduced NK cells activity. Two cases presented hypergammaglobulinemia with autoantibodies detected (anti-peroxidase and anti-tireoglobulin). Transitory neutropenia was found in 2 cases. Positivity sorology to citomegalovirus was found in 23/36 cases analysed, and there are no response to hepatitis B in 5/21 evaluated children. The data found in this study are valid for this specific population, although the immunodeficiencies research in DS patients should be done in the cases with maintenance of infections besides the appropriated control of associated diseases.



**Syndactyly type-I: study of four large Indian pedigrees.** *U. Radhakrishna<sup>1</sup>, V.K Patel<sup>1</sup>, M. Ravindra Babu<sup>1</sup>, U. Ratnamala<sup>1</sup>, J.V. Solanki<sup>2</sup>.* 1) Genetics, Green Cross Blood Bank & Genetics, Paldi, Ahmedabad, Gujarat, India; 2) Department of Animal Genetics & Breeding, Veterinary college, Gujarat Agriculture University, Anand, India.

Hereditary syndactyly was classified into five different types. Syndactyly type-I (SDTY1) (OMIM 185900) involves complete or partial bilateral syndactyly between third and fourth fingers which is occasionally associated with fusion of the distal phalanges. Feet are rarely affected. It may be an isolated condition. The genes responsible for SDTY1 and SDTY2 have been mapped to chromosome 2q34-q36 (Am J Hum Genet 67:492-97,2000 and 2q31(Hum Molec Genet 4:1453-1458,1995), respectively. We have studied four large Indian pedigrees with an autosomal dominant SDTY1. Pedigrees consist of 81 individuals, including 36 affected (20 males/16 females). Severity of the phenotype was quite variable among the families and no skipping of generation was observed. In three families, 12 members were bilaterally affected with typical features of syndactyly affecting the 3rd and 4th fingers and 10 members had only unilateral findings. Few of these also had unilateral partial syndactyly of 2nd and 3rd toes. In the fourth family, complete unilateral or bilateral syndactyly affecting 3rd, 4th and 5th fingers was observed. Few of the affecteds in this family had unilateral elongation of 2nd and 3rd toes with syndactyly. Phalangeal bones were not affected in any of these families, even though the nails are involved in few affecteds. Linkage studies with markers closely linked to SDTY1 and SDTY2 will either confirm allelism to these loci or provide evidence for genetic heterogeneity.

**Limb Reduction Defects In Aljahra Region of Kuwait(1983-1990):Prevalence and Clinical presentation.** *R.L. Al-Naggar<sup>1</sup>, S.A Al-Awadi<sup>1</sup>, S.A Madi<sup>2</sup>, M.M Abuhenedi<sup>1</sup>, T.I Farag<sup>1</sup>, L. Bastaki<sup>1</sup>.* 1) Obstetric&Gynecology, Kuwait Medical Genetics Center, Kuwait, Capital, Kuwait; 2) Pediatric Department, Aljahra Hospital,Kuwait.

Limb reduction defects(LRD)are of special interest,as they are the natural means of communications and possible indicators of environmental teratogens.Out of 60271 births,37 cases of LRD were ascertained.The data were collected from the Rigestry for congenital malformations of KMGC,Aljah.Hospital and patients clinical records.LRD represented 3.5% of total congenital anomalies and 18.7% of total limb malformations.The birth prevalence/10,000 births was 6.1 for total LRD,3.65 for isolated and 2.48 for cases with assaciated anomalies of other systems.The overall LRD had a high significant decline in 1989&1990 ( $P=0.01$ ),isolated cases also had a significant decrease ( $P=0.05$ ).LRD were classified into 9 categories,the most common LRD were terminal/transverse 24.4% and digital deficiency 16%,while the least LRD were rudimentary and postaxial defects,5.4%.The upper limbs were more affected than LL,54% vs 19%.The genetic causes were found in 66.7%,M:F ratio was1.8:1 and 64.8 of parents were blood relatives.There was also a significant increase of paternal age.The present study showed high prevalence of LRD,genetic factors were important added to that the advanced paternal age.We recommend further studies to evaluate the effect of mutagens/teratogens resulted from the 2nd Gulf War.

**Intestinal atresia, gallbladder agenesis/hypoplasia and severe IUGR in two siblings - A new autosomal recessive syndrome?** *B. Lo<sup>1</sup>, P. Shah<sup>2</sup>, R. Windrim<sup>3</sup>, S. Farrell<sup>4</sup>, K. Chong<sup>1</sup>*. 1) Div Clinical Genetics, Hospital for Sick Children, Toronto, ON., Canada; 2) Department of Neonatology, Hospital for Sick Children, Toronto, ON., Canada; 3) Division of Maternal-Fetal Medicine, Mount Sinai Hospital, Toronto, ON., Canada; 4) Department of Laboratory Medicine, Credit Valley Hospital, Mississauga, ON., Canada.

We describe here two siblings who presented with severe IUGR, gallbladder agenesis/hypoplasia, and multiple intestinal atresias. The first sibling was female and died of liver, renal and respiratory failure following surgery for repair of jejunal and pyloric atresias. Autopsy confirmed the aforementioned MCAs as well as a short pancreas and thymic atrophy. Postmortem cholangiogram revealed no gallbladder and two bile ducts draining separately into the pylorus and duodenum. The second sibling was male, born at 38 weeks gestation. Prenatally, intestinal atresia and IUGR were noted in the third trimester. Postnatally, during the surgical repair of the intestinal atresias, a hypoplastic gallbladder was noted. The siblings' features were not dysmorphic and their karyotypes were normal.

Review of the literature indicates that severe IUGR, intestinal atresia and gallbladder agenesis without biliary atresia has previously not been reported as a syndrome. Although Martinez-Frias et al (Am. J. Med. Gen., 1992) reported on two siblings with a similar pattern of gastrointestinal malformations, that syndrome included TEF, hypospadias, and extrahepatic biliary atresia, none of which were evident in our affected siblings. The MCA pattern reported here likely represents a new autosomal recessive syndrome. Prenatal diagnosis is possible when signs of IUGR, intestinal atresia and gallbladder agenesis/hypoplasia are present.

**Differential diagnosis and decision analysis in the management of low second trimester unconjugated estriol**

**(uE3).** *N.A. Hueppchen<sup>1</sup>, J. Hill<sup>1</sup>, C.J. Macri<sup>2</sup>.* 1) OB/GYN, National Naval Medical Center, Bethesda, MD; 2) Department of OB/GYN, USUHS, Bethesda, MD.

**BACKGROUND:** During the second trimester, alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG) and unconjugated estriol (uE3) in plasma are often measured as markers for fetal abnormalities. Several studies have shown that very low or undetectable levels of second trimester unconjugated estriol are associated with a variety of disorders, to include chromosomal abnormalities, steroid sulfatase deficiency, congenital adrenal hyperplasia, Smith-Lemli-Opitz syndrome (SLO), adrenocorticotropin deficiency, anencephaly, and miscarriages. **OBJECTIVE:** The purpose of this study was to examine the relationship between low maternal serum levels of uE3, sonographic findings, and adverse perinatal outcomes in series of pregnancies undergoing prenatal diagnosis in our Prenatal Assessment Center(PAC). Subsequently, we attempted to use this information to develop a management scheme to assist in the prenatal diagnosis of abnormal pregnancies identified by low maternal serum uE3 in the second trimester. **CASES:** Ten patients were identified who presented to the PAC with low maternal serum levels of uE3. The fetal biometry and sonographic findings were documented, and each patient was offered amniocentesis for karyotype and other biochemical testing as deemed necessary. The following conditions were diagnosed: aneuploidy, anencephaly, SLO, and X-linked ichthyosis. **CONCLUSION:** The finding of a very low or undetectable unconjugated estriol level on the second trimester maternal serum triple screen should be a warning sign, and prompt the clinician to additionally investigate for evidence of disorders, other than trisomy 18, for which biochemical or genetic testing is available.

**A nonsense mutation of *PVRL1* (W185X) is associated with non-syndromic cleft lip/palate in northern Venezuela.**

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Cleft lip ± cleft palate (CL/P) is among the most common of major birth defects, with an incidence of 0.4-2.0/1000. About 70% of cases are non-syndromic (nsCL/P), with complex inheritance. CL/P also occurs in many single-gene syndromes, and we have shown that autosomal recessive CLPED1 syndrome results from mutations of *PVRL1*, encoding a developmental cell-cell adhesion molecule. CLPED1 is common on Margarita Island, just north of Venezuela, due to homozygosity for a *PVRL1* nonsense mutation, W185X. On the island, ~1/26 normal persons carry the W185X mutation, many having a broad, flat upper lip, possibly a *form fruste* of CL/P. Further, the incidence of nsCL/P is very high on Margarita Island, ~5.4/1000. These findings suggested that the *PVRL1* W185X mutation might be a risk factor for nsCL/P in this region. We carried out a pilot case-control survey for the W185X mutation among patients with nsCL/P versus normals from Margarita Island, and found that 2 of 30 (q=.067) nsCL/P patients were W185X heterozygotes, versus 2 of 52 (q=.039) unrelated normals. This 1.7-fold increase, while suggestive, is not significant due to the high rate of heterozygosity among normals. Accordingly, we carried out a larger case-control study of the Cumaná region on the north coast of Venezuela, just south of Margarita Island, where the incidence of nsCL/P is also relatively high, ~2.5 per 1000. In Cumaná, 14 of 243 unrelated nsCL/P patients were heterozygous for the W185X mutation (q=.057), versus 1 of 245 unrelated normals from the region (q=.004) ( $P=.0004$ ). Further, the frequency of the W185X mutation among Cumaná nsCL/P patients (5.7%) was essentially the same as on Margarita Island (6.7%), suggesting that the degree of association between the mutation and nsCL/P is similar ( $P=0.691$ ) in these two populations. The *PVRL1* W185X mutation thus appears to be a significant, albeit moderate, genetic risk factor for nsCL/P in the Cumaná region of north-central Venezuela, the first thus far identified in any population.

**Distal 10q deletion and VUR.** *S. Sakazume*<sup>1,4</sup>, *Y. Kobayasi*<sup>4</sup>, *T. Nagai*<sup>4</sup>, *T. Ogata*<sup>2</sup>, *K. Muroya*<sup>2</sup>, *H. Ohashi*<sup>3</sup>, *Y. Fukushima*<sup>1</sup>. 1) Department of Hygiene & Med Genetics, Shinshu Univ, Matsumoto Nagano, Japan; 2) Department of Pediatrics, Tokyo Electric Power Company Hospital; 3) Division of Medical Genetics, Saitama Childrens Medical Center; 4) Department of Pediatrics, Dokkyo University School of Medicine Koshigaya hospital.

We report on a patient with chromosome 10q del. associated with VUR and a hypo/dysplastic kidney. The deleted site is a rather distal region than the reported genes, suggesting the existence of novel genes responsible for VUR in this region. The patient is a 3-year-old girl. She was born normally to a healthy 34y/o mother and a 30y/o father as a second baby. Her birth weight was 2358g. She showed several abnormal features, including mild hypotonia, micrognathia, low set ears and hypoplastic abdominal wall. At three month of age she developed UTI. Bilateral VUR (grade 5 and 3) complicated with right hypo/dysplastic kidney was diagnosed. Her karyotype, 46, XX, del (10) (q25.3). rev ish del (10) (q25.3) (WCP10+), was confirmed using G banding and FISH. The micro-satellite marker study confirmed that the paternal chromosomal region was deleted, and that the range of the deletion was from D10S587 to D10S215. There are several genes related to urinary tract formation, such as RET, PAX2, GFRA1 and EMX2, on chromosome 10. The patients break point is distal site of these genes. These genes are conserved in this patient. Ogata (2000) summarized that urinary tract abnormality is complicated more than 70% in the 10q del syndrome. It is possible that (an) unrecognized gene(s) responsible for VUR and urogenital anomalies is (are) located at the 10q terminal region.

**Pedigree Review to Determine Cancer-Related Deaths in Individuals with Neurofibromatosis type 1 in a Pediatric-based NF Clinic.** *K. Dent, B. Baty, J. Palumbos, S. Lewin, J. Carey, D. Viskochil.* Dept Pediatrics, Univ Utah Health Sci Ctr, Salt Lake City, UT.

Neurofibromatosis type 1 (NF1) is a progressive, multi-system disorder affecting approximately 1 in 3500 individuals worldwide. The recent report by Rasmussen et. al. (AJHG 2001) showing decreased life expectancy of individuals with NF1 led to our review of multi-generation NF1 pedigrees to identify cause of death in a Utah cohort. We focused on cancer-related deaths, specifically a common NF1-related cancer, malignant peripheral nerve sheath tumor (MPNST). MPNSTs are spindle cell sarcomas that often arise in the context of plexiform neurofibromas. The incidence of these tumors is estimated to be 2-5% in individuals with NF1, and they are detected most often in the 3rd or 4th decade of life. The 5-year survival rate is approximately 40 - 50%. We hypothesize that MPNSTs contribute significantly to the decreased life expectancy in NF1. We analyzed 155 individuals affected with NF1 within 20 three-generation families ascertained in the NF Clinic at the University of Utah. Only 19 deaths were reported. Twelve individuals (from 10 families) reported malignancies leading to death. Five of the 12 individuals experienced MPNSTs (3%) and 7 (4%) were described as having NF-related cancers including glioblastoma, intra-cranial tumor, lymphoma, and four of unspecified type. The remaining 7 deaths were not cancer related. These findings correlate with the expected incidence of MPNSTs in NF1 and support the conclusions by Rasmussen et. al. that malignancies of connective and other soft tissues occur more frequently than expected in individuals who die of NF1. Upon review of the pedigrees of these 20 families and several two-generation families, we recognized the potential for under-ascertainment of cancer-related deaths in NF1, specifically MPNSTs. To address this issue, providers of genetic services must routinely ask about causes of death and history of cancer when obtaining family history information. Longitudinal studies and more accurate collection of causes of death in extended pedigrees are needed to determine the true incidence of MPNST-related deaths in NF1.

**A mild phenotype of Incontinentia Pigmenti in a male child: DNA confirmation of a somatic mosaicism. S.**

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Familial Incontinentia Pigmenti (IP) is an X-linked dominant genodermatosis, lethal in males. Affected females demonstrate abnormalities of the skin, teeth, and central nervous system. Eighty-five percent of IP cases are due to a gene rearrangement within the NEMO gene which deletes exons 4-10. We describe an affected male showing an IP female-like phenotype who was born after a normal pregnancy with a birth weight of 2670 g. Family history was negative for skin and neurologic disorders, IP and abortions. At birth, erythematous blisters scattered over the body surface, according to the distribution of Blaschko lines. At 10 days of life, a skin biopsy was consistent with IP. The standard karyotype was 46, XY, normal. At 30 days of life, some warty lesions were evident on the hands. At 5 months of age, some linear yellowish streaks were present in the areas previously affected by vesicular lesions. Now, at 23 months of age, the child is growing well, shows very light yellowish streaks on the skin, has a conic tooth in his mouth and has a normal neurological development. DNA from patient's peripheral blood lymphocytes was analysed for the presence of NEMO gene deletion of exons 4-10, using a previously established protocol. The analysis showed the identical gene deletion carried by most of the IP patients. Moreover, using other specific primers for the wild type NEMO gene, we detected the usual band of the normal allele as well. Parents' DNA was negative for gene rearrangement. These results suggest an early postzygotic mutation ending in a possible somatic mosaicism, which explains the survival and mild phenotype of our male patient. Further molecular tests are in progress to show the mosaicism rate in tissues other than blood. Our case report provides a possible genetic mechanism for X-linked dominant disorders in surviving males.



**DNA evaluation of a Marfan syndrome mutation with high phenotypic variability and the counseling issues about heart disease.** *F.V. Schaefer<sup>1</sup>, P. O'Laigue<sup>2</sup>, L. Whetsell<sup>1</sup>, C. Curry<sup>2</sup>*. 1) Dept Molecular Genetics, HA Chapman Inst Medical Gen, Tulsa, OK; 2) Genetic Medicine Department, Valley Children's Hospital, Maderia, CA.

A large percentage of patients who undergo direct DNA testing at the HA Chapman Institute present with possible Marfan syndrome but do not fulfill sufficient clinical criterion nor have sufficient family history to be definitively diagnosed. However, correct diagnosis is critically important due to the life-threatening aspects of Marfan syndrome, heart disease. This family illustrates a compounding difficulty of DNA diagnosis in Marfan syndrome and the protocol that we use in order to pursue the clinical significance. In contrast to most patients we receive for testing, this family had several cases of diagnosed Marfan syndrome. An Ala882Thr gene alteration was discovered but there was a concern as to its clinical significance. Consequently, 7 other family members were examined and the mutation was identified in 3 with a Marfan diagnosis and not found in 3 members without the Ala882Thr change. However, one family member with no phenotypic signs was also found with the DNA alteration. As a result of the DNA studies, the counselor made a recommendation for baseline heart and eye studies to be performed on all 3 offspring (all in their 20s) of the proband including the daughter without any phenotypic signs of Marfan syndrome. During the baseline exam this daughter was discovered to be undergoing aortic dissection and was operated on the day after the exam. On balance, the evidence suggests that the Ala882Thr alteration is a mutation. Most importantly, however, it demonstrates that a life-threatening heart problem can occur in an individual with no outward phenotypic signs of Marfan syndrome. Without the DNA testing and the resulting echocardiogram the consequences of the daughters dissecting aorta could have been fatal. Combined with our previous survey data on our cases, this family illustrates that the heart involvement the major life-threatening aspect of the disease - may be the common denominator in most cases and DNA analysis is an especially important part of the clinical evaluation.

**A Clinical and molecular study of 20 German patients with Fibrodysplasia Ossificans Progressiva. G.G.**

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Fibrodysplasia ossificans progressiva (FOP) is a rare disorder of connective tissue where there is progressive ectopic ossification and characteristic skeletal malformations. The FOP phenotype has previously been linked to markers located in the 4q27-31 region. We report on the clinical and molecular findings in 20 German patients with FOP (9 male, 11 female) with an age ranging from 3 to 38 years. All were sporadic cases. The patients were examined personally (CG, GG-K) and had to answer a questionnaire. Ectopic ossification started on average at 3.8 years (birth to 11 years) leading to extra-articular bony ankylosis of nearly all joints of the axial and appendicular skeleton. Hypoplasia of big toes was present in 12/20 patients, 10 patients had a hypoplastic thumb. Hearing loss was found in 7/20 individuals. Average paternal age was 30.6 years (19-40y). There are a number of interesting candidate genes in 4q27-q31 such as *FGF-2* (Fibroblast-Growth Factor 2), *EGF* (Epidermal Growth factor), *SMAD-1*, *IL-15*, *NFκ-B1* and others. We performed mutational analysis in the Toll-like receptor 2 (*TLR2*) but were unable to find any mutations. A further search for mutations will help to understand the biological pathway of the disease and help to provide evidence for future treatment.

**Evidence for a common ancestor in 2 large families with phenotypically variable spastic ataxia.** *K.K. Grewal<sup>1</sup>, M.G. Stefanelli<sup>1</sup>, I.A. Meijer<sup>2</sup>, C.K. Hand<sup>2</sup>, G.A. Rouleau<sup>2</sup>, E.J. Ives<sup>1</sup>.* 1) Discipline of Medical Genetics and Division of Neurology, Faculty of Medicine, Health Sciences Centre, Memorial University, St. John's, Newfoundland, A1B 3V6 Canada; 2) Centre for research in Neuroscience, McGill University, Montreal General Hospital research Institute, Montreal, H3G 1A4 Canada.

A slowly progressive neurodegenerative disorder in which both spasticity and ataxia are common features is described in 2 large independently ascertained Newfoundland families, family 71 and family 13. Despite the marked phenotypic diversity, clinical similarities exist between these families and both pedigrees show linkage to a new locus which includes a 13.6cM haplotype shared by all affected members. Family 71 has 16 clinically affected living members (ages 22-76 yrs) and family 13 has 34 (ages 21-71 yrs). Segregation and sex ratios are consistent with autosomal dominant inheritance. The disorder is difficult to classify but often presents with hypertonicity, hyperreflexia and eye movement abnormalities and could be termed a spastic ataxia. The range of expression is very variable in both families with some members exhibiting mild symptoms whilst others have a broader more severe phenotype even within the same sibship. Onset for most cases occurs within the first 2 decades with leg spasticity, clumsiness and an involuntary head jerk recognised by family members. More difficulties are encountered by the third decade with progression of spasticity, significant dysarthria with characteristic scanning nasal speech and intermittent dysphagia. Supranuclear gaze palsy, dystonia and proprioceptive errors are also observed. By the fourth decade the broad-based spastic and ataxic gait creates major problems for independent mobility but neither cognition nor life span are noticeably affected. There is no evidence for a sex influence on the phenotype. Families 71 and 13 both originate in a rural historically isolated geographic area of the island of Newfoundland where it is presumed that the occurrence of the condition reflects a common ancestor. The disorder contributes disproportionately to the burden of adult neurological degenerative disease in the area and identification of the specific mutation will facilitate appropriate genetic testing.

**Ectodermal dysplasia syndrome with mental deficiency, joint laxity, nystagmus, cerebellar ataxia, periventricular nodular heterotopia, osteopenia and hyperglycinuria.** *R. Zannolli*<sup>1</sup>, *C. Miracco*<sup>2</sup>, *M.M. de Santi*<sup>2</sup>, *G. Vatti*<sup>3</sup>, *P. Galluzzi*<sup>5</sup>, *A. Malandrini*<sup>4</sup>, *E. Conversano*<sup>1</sup>, *L. Serracca*<sup>1</sup>, *M. Molinelli*<sup>1</sup>, *G. Coviello*<sup>6</sup>, *P. Terrosi-Vagnoli*<sup>7</sup>, *S. Gonnelli*<sup>8</sup>. 1) Dept of Pediatrics, Obstetrics, and Reproductive Medicine, Section of Pediatrics, Policlinico le Scotte, University of Siena, Siena, Italy; 2) Institute of Pathological Anatomy and Histology, Policlinico le Scotte, University of Siena, Siena, Italy; 3) Dept of Neurosciences, Section of Neurology, Policlinico le Scotte, University of Siena, Siena, Italy; 4) Institute of Neurological Sciences, Policlinico le Scotte, University of Siena, Siena, Italy; 5) Neuroradiology Unit, Azienda Ospedaliera Senese, Policlinico le Scotte, Siena, Italy; 6) Radiology Unit, Azienda Ospedaliera Senese, Policlinico Le Scotte, Siena, Italy; 7) Dept of Radiology and Orthopedics, Policlinico Le Scotte, University of Siena, Siena, Italy; 8) Institute of Internal Medicine, Policlinico le Scotte, University of Siena, Siena, Italy.

We may be seeing a novel ectodermal dysplasia (ED) syndrome with a pseudo-cranioectodermal dysplasia phenotype (MIM 218330) associated with a wide spectrum of features (mental deficiency, joint laxity, cerebellar ataxia, periventricular nodular heterotopia, osteopenia and hyperglycinuria). The proposita is a seven-year-old girl, the second child born from consanguineous parents (first cousins born from two of five siblings). The older sister, 13 years old, was referred as being apparently normal. The mother, apparently healthy and mentally unimpaired, had a subtle set of ED traits (hair, teeth and minimal skeletal dysmorphisms). The father was described as a normal healthy man with minimal hair and teeth problems. Both the proband's sister and the parents had hyperglycinuria. Of interest, several other members of both the mother's and father's family have suffered from thin and sparse hairs with subtle areas of alopecia, dental health failure and/or hyperglycinuria.

**Limb reduction defect associated with  $\alpha$ -thalassemia homozygous state.** *M. Thomas<sup>1</sup>, D. Myles Reid<sup>1</sup>, D. Chui<sup>2</sup>, D. Chitayat<sup>1</sup>*. 1) The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; 2) Provincial Hemoglobinopathy DNA Laboratory, Chedoke-McMaster Hospital, Hamilton, Ontario, Canada.

The association between limb reduction defect and  $\alpha$ -thalassemia is rare but has been reported before. The etiology of this association has not been delineated. We report a pregnancy who presented with limb reduction defect and hydrops fetalis and who was found to have  $\alpha$ -thalassemia homozygous state. The parents were of Filipino origin. The couple has one healthy daughter and this was their second pregnancy. The mother presented at 22.8 weeks gestation with fetal ultrasound findings of hydrops fetalis, oligohydramnios, pleural effusion, severe cardiomegaly, missing fingers on left hand and missing toes on both feet. Hematological investigation revealed that both parents were heterozygous for  $\alpha$ -thalassemia. The mother was a carrier of the Filipino  $\alpha$ -thalassemia-1 deletion (--FIL/aa) mutation and the father was a carrier of the southeast Asian  $\alpha$ -thalassemia-1 mutation (--SEA/aa). DNA analysis showed that the fetus was compound heterozygote for the Filipino  $\alpha$ -thalassemia-1 deletion and the southeast Asian  $\alpha$ -thalassemia-1 mutation (--FIL /--SEA). The pregnancy was terminated and the autopsy confirmed the fetal ultrasound findings including hepatosplenomegaly and extensive extramedullary hematopoiesis. Advances in antenatal diagnosis, intrauterine intervention, and postnatal treatments have resulted in possible survival of fetuses with alpha thalassemia homozygous. When the parents consider prenatal treatment with fetal blood transfusion a detailed fetal ultrasound including detailed survey of fetal limbs is mandatory and discussion of limb reduction defects should be included in the genetic counselling.

**Mechanism of cardiac malformations in patients with bilateral pulmonary agenesis.** *R.S. Colby, R.E. Stevenson, L.H. Seaver, N.R. Cooley, Jr.* Greenwood Genetic Ctr, Greenwood, SC.

Pulmonary agenesis is defined by complete absence of the bronchus and all lung tissue below the bifurcation of the trachea. It can occur as a unilateral or bilateral defect. Bilateral pulmonary agenesis (BPA) is rare, reported in 18 patients. BPA is associated with other malformations and is always associated with specific cardiovascular malformations. The cardiovascular structures affected are those related to pulmonary perfusion. All cases have had agenesis of the pulmonary veins and the pulmonary artery is agenic or joins the aorta at the ductus without giving off branches. A fetal autopsy at the Greenwood Genetic Center with BPA confirmed these associations. A dizygotic twin born at 35 weeks showed agenesis of the pulmonary valve, artery, and veins, agenesis of the ductus arteriosus, and a VSD with an overriding aorta. Other malformations included bilateral microphthalmia, GI malformations, and GU malformations. The cotwin was normal.

The laryngo-tracheal diverticulum develops as a ventral outgrowth from the foregut just caudal to the pharynx. Lung buds develop from this structure at the end of the fourth week. Angioblasts proliferate around the lung buds and form the primordium of the common pulmonary vein. Likewise a pulmonary vascular plexus forms around the developing lung buds and eventually connects to the pulmonary arteries that come from the sixth aortic arch.

If the lung buds or vascular plexuses around the lung buds fail to form then the entire architecture of the heart destined to perfuse the lungs will also fail to form. The heart may not have the typical divisions, the pulmonary veins and arteries will not form, and the truncus arteriosus may not divide. This results in the cardiac malformations seen with BPA.

BPA is associated with other malformations. These include anophthalmia/microphthalmia, gastrointestinal anomalies, and genitourinary anomalies. Many of these structures begin to form around the fourth week of development, suggesting a common mechanism acting during this period. A time limited defect in angiogenesis is a possible explanation.

**Molecular confirmation of somatic mosaicism in a karyotypically normal male with X-linked dominant chondrodysplasia punctata 2 (CDPX2).** *D.J. Aughton*<sup>1</sup>, *R.I. Kelley*<sup>2</sup>, *A. Metzenberg*<sup>3</sup>, *R.M. Pauli*<sup>4</sup>. 1) Wm. Beaumont Hospital, Royal Oak, MI; 2) Kennedy Krieger Institute, Baltimore, MD; 3) California State U.-Northridge, CA; 4) U. of Wisconsin-Madison, WI.

CDPX2 (Conradi-Hünemann syndrome [McK #302960]), an X-linked dominant condition lethal in hemizygous males, is characterized by epiphyseal stippling, skin changes that follow Blaschko's lines, patchy alopecia, and short stature. It is associated with increased levels of 8-dehydrocholesterol and 8(9)-cholestenol; it is caused by abnormal function of *EBP*, which maps to Xp11.22-p11.23. In 1995, Happle proposed either a postzygotic mutation or a gametic half-chromatid mutation to explain the observation of CDPX2 in 3 46,XY males. Both gonadal and somatic mosaicism have been described in CDPX2, but somatic mosaicism in a karyotypically normal male with CDPX2 has not previously been demonstrated. We report such a case.

A 6-month-old boy presented with macrocephaly, mild scoliosis, and equivocally short limbs. Skeletal survey showed punctate epiphyseal calcifications, scoliosis, butterfly vertebræ, and mild long bone shortening. Examinations through the first 3 years of life also showed facial characteristics of CDPX2, spotty alopecia, linear hypotrophic skin lesions, and mild asymmetry of the legs and feet, findings consistent with a diagnosis of CDPX2. Additional studies included routine chromosome analysis (46,XY), plasma sterol analysis (8[9]-cholestenol and 8-dehydrocholesterol), and FISH analysis of sex chromosomes in buccal cells (all XY). Molecular analysis of *EBP* demonstrated 2 populations of cells, 1 with the wild-type allele and 1 with an altered allele (G238A, E80K) often seen in affected females. Clinical, biochemical, and molecular assessment of the boy's mother showed macrocephaly but no other evidence of CDPX2.

The proband therefore has mosaicism for a *de novo* mutation of *EBP*. His linear skin lesions, spotty alopecia, and asymmetries thus arose from somatic mosaicism rather than X inactivation. His case provides independent support for the Happle hypothesis.

**Lymphedema, unique facial features and tapered fingers in three females in three generations: A new lymphedema/multiple congenital anomaly syndrome or an expanded phenotype of the lymphedema/distichiasis syndrome?** *R.G. Cadle, B.D. Hall.* Dept Pediatrics/Genetics, Univ Kentucky Medical Ctr, Lexington, KY.

There are several syndromes partially characterized by lower extremity lymphedema, including Milroys disease, and the less common Hennekam syndrome and lymphedema/distichiasis syndrome (LD). We report a mother, daughter and grand daughter with a lymphedema/multiple congenital anomaly syndrome.

The proband, ED, was a 16-year-old pregnant Caucasian female with unique facial features and lymphedema of the lower extremities which was first noted at 14 years of age. EDs mother, JD, developed lymphedema of the legs at approximately ten years of age. Both ED and JD have a full, broad nasal tip, wide nasal bridge, dystopia, long, simple philtrum, broad neck, and tapered fingers. JD has developed pes cavus with age. Both ED and JD have chronic red and irritated eyes of unknown etiology. Both have had numerous negative allergy evaluations. Neither has had a formal eye exam, but both have disorganized eyelashes, which may be the cause of their eye irritation. Distichiasis has not been confirmed. We evaluated EDs baby, AD, at five weeks of age. She had epicanthal folds, hypertelorism, broad nasal bridge, prominent pre-maxilla, redundant skin on the nape of the neck and mildly tapered fingers. No lower extremity edema was noted.

A skeletal survey of JD indicated no major bone abnormalities. FISH analysis (done by Dr. Thomas Glover at the University of Michigan) did not detect a microdeletion in FOXC2, mutations in which have recently been found to cause LD. However, based on the age of onset of the lower extremity lymphedema in our family, and the suspicion that there are abnormalities of the eyelashes, the possibility of LD is considered. Patients with LD, though, do not share the facial dysmorphology noted in our three patients. Further molecular testing will be necessary to better determine whether or not our family has a new mutation in the FOXC2 gene or whether they represent a new lymphedema/multiple congenital anomaly syndrome.



**OSA syndrome (ocular, skeletal, and abdominal abnormalities): Confirmation of the autosomal recessive syndrome in a sporadic male.** *B. Bender, B.D. Hall.* Department of Pediatrics, University of Kentucky, Lexington, KY.

In 1996, Mingarelli, et al, described two adult sisters with a unique pattern of ocular, facial, skeletal, and abdominal abnormalities including short, downslanting palpebral fissures and ptosis with thin high-arched eyebrows, high nasal bridge, flat malar bones, high-arched palate, and diastasis recti. One sister had limitation to supination secondary to radioulnar synostosis, while the other had carpal fusion. The older sister also demonstrated aortic incompetence. In addition, the two girls each had lumbosacral spina bifida and normal intellectual functioning. (*J Med Genet* 1996; 33:884-886) We report an 11-year-old boy with remarkably similar facial features, including the very distinctive high-arched eyebrows and flat malar bones, who originally presented at 6 months of age. He is the full-term product of a non-consanguineous, uncomplicated pregnancy to a 33-year-old G2P1 mother. Birth weight was 8 pounds, 14 ounces and birth length was 20 1/2 inches. He was noted at birth to have a sacral appendage and a small mid-sternal skin tag. Other unique features include a PDA that was ligated at 2 years of age, inability to supinate past the neutral position, and a lumbosacral dysraphism (L4 to S2). Our patient did not have a diastasis recti. He has normal psychomotor development. Chromosome analysis revealed a pericentric inversion on the Y, which his father also carries; consequently, it is thought to be unrelated to these anomalies. The similarity between our patient and those reported by Mingarelli, et al. is impressive, and we conclude that this is indeed a distinct entity. The distinctive eyebrow configuration with additional dysmorphism is a good indicator to consider this syndrome.

**Redefining a case of Jarcho-Levin Syndrome as Oculo-Auriculo-Vertebral Spectrum.** *S.F. Cheng<sup>1</sup>, G.M. Enns<sup>2</sup>, M. Golabi<sup>1</sup>, B. Blumberg<sup>1</sup>, D.R. Kostiner<sup>1</sup>.* 1) Dept Pediatrics/Div Genetics, UC San Francisco, CA; 2) Dept Pediatrics/Div Genetics, Stanford University, CA.

OAV spectrum is a condition typified by hemifacial microsomia, lipodermoids, facial skin tags, ear anomalies and vertebral defects. Less common features include anal atresia and defects of the heart, lungs, kidneys and CNS. Many features of OAV overlap those of VATER and CHARGE. Discordant presentation of OAV in twins has been documented. Jarcho-Levin syndrome (JLS) is a type of short trunk dwarfism characterized by crab-like thorax, infant mortality and autosomal recessive (AR) inheritance. We present the case of a 5 y.o. boy diagnosed with JLS as a neonate whose features are now more consistent with the severe end of OAV spectrum. Assuming this child truly has OAV, the recurrence risk for his parents decreases from 25% (AR) to 2-3% (sporadic). The proband and his unaffected same-sex twin were born at 33 weeks to a 29 y.o. woman. Newborn exam revealed asymmetric ears, unilateral ear tags, short neck, misshapen chest, anal atresia, GU anomalies and camptodactyly. Karyotype showed 46,XY with no 22q deletion. X-rays showed various vertebral defects, fused ribs and crab-like thorax. A presumptive diagnosis of JLS was made. Annual follow-up for 5 years revealed developmental delay, short stature, lipodermoids and some improvement in thoracic defects. The diagnosis of JLS was questioned based on the child's hemifacial microsomia and improvement with time. Literature review revealed that Mortier et al. (1996) reclassified JLS into 4 categories, one of which describes sporadic inheritance, various thoracic defects, additional structural defects (craniofacial anomalies, anal atresia) and survival beyond childhood. Features of this category overlap those seen in severe OAV, suggesting the two may be in the same spectrum. Furthermore, since features of OAV, VATER and CHARGE overlap, there is growing consensus that all 3 are in the same spectrum. Variations in presentation are likely due to genetic heterogeneity and intrauterine factors (such as twinning). Longitudinal follow-up is helpful in genetic diagnosis, since reassignment of diagnosis can change recurrence risks for families.

**A new autosomal dominant syndrome of dermal ridge hypoplasia and palpebral hypertrichosis.** *J. Doucet<sup>1</sup>, A. Lirette<sup>1</sup>, S.M. Robichaux<sup>1</sup>, Y. Lacassie<sup>2</sup>*. 1) Dept Biological Sci, Nicholls State University, Thibodaux, LA; 2) Dept Pediatrics, LSU Health Sciences Center, New Orleans, LA.

We report a large kindred expressing a constellation of dermal abnormalities that describe a new syndrome. The features of the syndrome include dermal ridges hypoplasia of both the palmar and plantar surfaces, distichiasis and hypertrichotic growth across the eyelids as the brows thicken and descend toward the lateral canthi, raised and pyramidal fingerpads and toepads, under-keratinized and sweaty palmar and plantar surfaces, dorsal hyperpigmentation of the phalangeal surfaces, excessive tearing of the eyes, and coarse body hair. The syndrome affects four generations of an extended southern Louisiana family of Acadian descent and is inherited in an autosomal dominant fashion.

**Robert's-SC phocomelia Syndrome in two Infants with Different Ethnicity in Kuwait : Brief clinical report.**

*M. Mostafa, Henedi, S.A Alawadi, L. Bastaki, A. Alwadani, K. Murthy, R.L Alnaggar.* Obstetric&Gynecology, Kuwait Medical Genetics Center, Kuwait, Capital, Kuwait.

We describe two cases with tetraphocomelia/unusual facial features and cytogenetic findings in Pakistani& Kuwaiti boys. The described phenotype is consistent with Robert's-SC phocomelia. We did a comparison between ours and the previously reported. The 1st case was Pakistani, newborn and preterm baby having oxycephaly, sparse/silvery hair, shallow orbit, exophthalmos, ectropion, cloudy corneas, bilateral cleft lip/palate, hypoplastic ears and facial hemangioma. There were symmetrical limb reduction defects, flexion/contracture of joints and ambiguous genitalia. Skeletal survey revealed hypoplastic joints, deficient carpal/metacarpal bones, adactylia/aphalandia in both hands, rudimentary 12th rib, short/deformed tibiae, absent left fibula and deficient metatarsal/phalanges in both feet. Karyotype showed absence of premature separation of centromeres. The 2nd case was 1 year and 8 months old, Bedouin boy having IUGR and postnatal growth delay. His phenotype included facial capillary hemangioma, short nose, dysplastic ears retrognathia, short neck and pectus excavatum. Upper limbs showed bilateral phocomelia, short hands, oligodactylia/aphalangia and dysplastic nails/skin with dysplastic creases. Bilateral hip joints hypoplasia/dislocation, knee aplasia, ankle hypoplasia, phocomelia, absent fibulae, deficient metatarsals and oligodactylia have been found in lower limbs. External genitalia showed pigmented, empty scrotum and macrophallus. Skeletal survey revealed short humeri, bilateral humeroradial fusion, hypoplastic radii, absent ulnae, absent/deficient carpal/metacarpal bones and aphalangia. Hypoplastic hip bones, shallow acetabulum, short/angulated and dysplastic femora, short tibiae absent fibula/tarsal, metatarsal bones with aphalangia. Cytogenetic & FISH studies revealed balanced, reciprocal translocation,  $t(1q:3q)(q32-q21)$ , like his normal daughter transmitted from the father. Both phenotypes share in common the severe symmetric limb reduction defects, recessive inheritance and absence of premature separation of centromere, but slightly differ in some craniofacial features. These two cases could be allelic variants?.

**Paternal UPD 14 unrelated to translocation.** *E. McPherson*<sup>1,2</sup>, *R. Pinter*<sup>1</sup>, *S. Parida*<sup>2</sup>. 1) Dept Genetics, Magee Womens Hosp, Pittsburgh, PA; 2) Dept Pediatrics, Childrens Hosp of Pittsburgh.

Paternal uniparental disomy for chromosome 14 (pat UPD 14) is associated with a recognizable phenotype including narrow thorax, short limbs, webbed neck, contractures and characteristic facial appearance (short palpebral fissures, broad nose, prominent philtrum, small posteriorly rotated ears). Only 4 cases have been reported, all with translocations or isochromosomes involving 14q. We report a newborn with mildly short limbs and small thorax, who was referred because of suspected Jeunes Asphyxiating Dystrophy, but was noted to have additional anomalies including short palpebral fissures, broad nasal bridge, prominent philtrum, posteriorly rotated ears, excess nuchal skin, hypospadias. The mother had been followed prenatally for polyhydramnios and ultrasound had shown short fetal limbs. The karyotype at amniocentesis was 46XY. The fetus was born at 35 weeks with weight 2166g (40%), length 43cm (5%) and OFC 32cm (50%). His chest was extremely small (circumference 23 cm) and he had mild rhizomelic shortening of his limbs. X-rays confirmed short ribs, and short limbs, but there was no generalized skeletal dysplasia and the spine and pelvis appeared normal. The normal 46 XY karyotype was confirmed in lymphocytes. Because of clinical resemblance to reported UPD 14 patients, uniparental disomy studies were obtained and the child was found to have paternal isodisomy 14. He had a difficult clinical course due to chronic respiratory insufficiency and died at 3 months during a respiratory infection. To our knowledge, this is the first case of pat UPD 14 unrelated to a translocation. The possible mechanisms are non-disjunction in paternal meiosis 2 with subsequent trisomy rescue or maternal non-disjunction with monosomy rescue by duplication of the paternal 14. These events may be more common than previously recognized. UPD 14 studies are recommended for children who have thoracic dystrophy and short limbs with additional anomalies not expected in classical Jeune syndrome.

**A familial t(6;22) translocation resulting in both Duane syndrome and Velo-Cardio-Facial syndrome in siblings.**

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Balanced translocation carriers may bear a high risk of having abnormal children with different dysmorphic features. We report a t(6;22) in a family where the two brothers each inherited a different derivative chromosome from their phenotypically normal father who carried a balanced t(6;22). The proband was a 41-year-old male and was referred for chromosome study as part of the evaluation of his wife's recurrent pregnancy loss. His history was significant for Duane retraction anomaly, absence of one kidney and oligospermia. Cytogenetic study revealed that the proband had a supernumerary marker chromosome. Fluorescence in situ hybridization (FISH) demonstrated that the marker was derived from chromosome 22 and contained the DiGeorge syndrome chromosome region (DGCR). Hence, he had three copies of 22pter@q11. Parental studies discovered that the father carried a balanced translocation of t(6;22)(p25;q11). FISH studies proved that the breakpoint on the chromosome 22 was distal to the DGCR. The proband's only sibling was a 35-year-old male with signs of Velo-Cardio-Facial syndrome (VCFS) and azoospermia. His history was noticeable for nasal speech, frequent infection, developmental delay and learning difficulties. A testicular biopsy in evaluation of infertility showed absence of sperm. Cytogenetic studies exhibited a karyotype of 45,XY,der(6)t(6;22)(p25;q11),-22. FISH studies found that the der(6) did not contain the DGCR as was seen in his father; therefore, he is monosomic for the DGCR. Two previous cases of Duane syndrome associated with marker chromosome derived from chromosome 22pter@q11 were reported. In both cases, the marker was bisatellited, indicating the presence of four copies of the 22pter@q11 region. These findings suggest that a gene or genes located in the region of 22pter@q11 may be associated with Duane syndrome and the development of the urogenital system. In addition to the features of VCFS, the proband's brother had azoospermia. Since most of the VCFS patients of reproductive age are fertile, the azoospermia may have been caused by the deletion of band 6p25.

**Application of GenDis (an original computer software) for diagnosis of malformation syndromes.** *K. Naritomi<sup>1</sup>, T. Tohma<sup>2</sup>*. 1) Dept Medical Genetics, Univ Ryukyus Sch Medicine, Nishiraha, Okinawa, Japan; 2) Dept Pediatr, Univ Ryukyus Sch Medicine, Nishiraha, Okinawa, Japan.

UR-DBMS (University of the Ryukyus-Database for Malformation Syndromes) is an original database created and collected data since 1986, mainly from well-known English written textbooks, O-MIM and magazines. The latest version 8 includes clinical and molecular genetic informations of more than 6,500 genetic diseases and genes (total volume is about 700 MB). This year, UR-DBMS Ver. 8 has developed to a computerized diagnostic software (GenDis). Following an input of all findings of the patient, the GenDis automatically selects candidate syndromes in order of increasing matched number of findings. This GenDis was applied to 393 pooled patients with multiple congenital anomalies for the diagnosis, and tested its usefulness. In 141 patients (36%), tentative diagnoses were confirmed. In 111 patients (28%), an appropriate diagnosis was given by GenDis. In 66 patients, a diagnosis was suggested but not conclusive with some questions. In 75 patients, an appropriate diagnosis was not suggested, mainly due to lack of critical data and clinical information, or an excess of candidates. Two patients were considered to have a broadened spectrum of known syndromes. As a whole, appropriate diagnosis was suggested in 64% of the patients tested. Thus, application of the GenDis is considered useful diagnostic tool in clinical genetics for quick diagnosis and further examinations to confirm.

**Autosomal Dominant Robinow Syndrome in 11 Affected Family Members: Marked Phenotypic Variability.** *D.*

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Robinow syndrome (RS) is a genetic disorder exhibiting both autosomal dominant and recessive modes of inheritance. Typical features include short stature, hypertelorism, cleft lip/palate, small hands, rib/vertebral defects, renal defects, genital abnormalities and normal intelligence. We report on a male baby with multiple anomalies who has 10 maternal relatives with similar but less severe phenotypes. We propose that all 11 members have RS and that the proband also has diabetic embryopathy. The proband was born to a 27 yo G2P1-2 mother at 34 weeks. Pregnancy was complicated by gestational diabetes treated with insulin starting at 26 weeks. Birth WT, HT, and HC were all ~40%. Multiple anomalies were noted at birth, including marked hypertelorism, facial dysmorphism, bilateral cleft lip/palate, dextrocardia, rib/vertebral anomalies, tethered cord, 2-vessel cord, single kidney, posterior urethral valves and cryptorchidism. Later complications included laryngotracheomalacia, obstructive apnea, respiratory arrests, inguinal hernias, fibrous subvalvar LV outflow obstruction, and multiple infections. At age 19 months, HT and HC were < 3%, WT was 15%, dysmorphism was striking and development was 2 months delayed. The proband's mother has short stature, hypertelorism and short fingers. Her father has similar features plus cleft lip/palate, and her grandmother had similar features plus 3 kidneys. Seven other family members have striking hypertelorism. Intelligence of all members is normal. This child's multiple anomalies are consistent with RS. The presence of 10 relatives with less severe phenotypes suggests dominant inheritance with marked variability in expression. The proband's dextrocardia and tethered cord are likely related to teratogenic effects of maternal diabetes and illustrate the role that the intrauterine environment may play in altering phenotype in genetic disorders. Data from this family imply that fitness may not be markedly reduced in autosomal dominant RS. The genes for RS have not been identified, but linkage analysis on this family might help in their discovery.



**Primary Hypothyroidism and Osteoporosis in Neuhauser Syndrome.** A. Sarkozy<sup>1</sup>, R. Mingarelli<sup>1</sup>, F. Brancati<sup>1</sup>, B. Dallapiccola<sup>1,2</sup>. 1) CSS-Mendel Institute, Rome, Italy; 2) Department of Molecular Medicine and Pathology, University La Sapienza, Rome, Italy.

Megalocornea-Mental Retardation (MMR) or Neuhauser Syndrome, is a rare autosomal recessive disorder first reported in 1975 by Neuhauser in nine patients. We examined an Italian 18 month old female child with MMR. Development milestones were delayed in the first months and myoclonic seizures became manifest at 8 months. She presented as a well-built brown girl, with head circumference of 41.8 cm (> -2 SD), weight 10.3 kg (25th centile), and length 77 cm (10th -25th centile). Main features were megalocornea, microcephaly, hypertelorism, bilateral epicanthal folds, micrognathia and asymmetric ears. Clinical examinations disclosed primary hypothyroidism, osteoporosis and hypercholesterolemia. The karyotype was normal. The clinical outline of MMR syndrome is tricky, because of the wide clinical variability. Comprehensive review of published MMR patients indicates that mental and growth retardation are invariably present, megalocornea occurs in 25 of 29 patients (86%), while a number of features recorded in MMR are found only in individual patients. This child showed the features shared by the majority of MMR subjects. In addition, she had primary hypothyroidism and osteoporosis, which were previously recorded only in individual patients. Hypercholesterolemia, which was likely related to hypothyroidism, has not been previously associated to MMR. Recurrence of thyroid and bone defects is arguing for a non random association with this disorder, suggesting that these anomalies are additional features of Neuhauser syndrome.

**Congenital Generalized lipodystrophy of Berardinelli-Seip : a study of 47 patients belonging to 9q34 and non-9q34 subgroups.** *L. Van Maldergem<sup>1</sup>, J. Magre<sup>2</sup>, E. Khallouf<sup>3</sup>, T. Gedde-Dahl Jr<sup>4</sup>, and the Berardinelli-Seip study group<sup>1</sup>.* 1) Centre Genetique Humaine, Inst Pathology & Genetics, Loverval, Belgium; 2) Inserm U 402, Faculte de Medecine Saint Antoine, Paris, France; 3) Institue of Forensic Medicine and Dept of Pediatrics, Rijkshospitalet, Oslo, Norway; 4) Service de pediatrie, Hotel-Dieu de France, Beyrut, Lebanon.

A series of patients with congenital generalized lipodystrophy, belonging to two groups designated according to their disease-causing mutation or their assigned locus after linkage study have been studied through an international consortium. We carried out a comparison between 9q34 and non-9q34. No genotype-phenotype correlation emerged and no distinctive phenotypic relationship could be distinguished between the two major loci. By contrast, although lipodystrophy was present from birth in a majority of affected patients, severe and mild phenotypes were observed with some patients coming to medical attention only in their second or third decades. The latter finding raises questions on the generally accepted classification between so-called congenital and acquired forms of generalized lipodystrophy. Facial lipodystrophy was less pronounced in infancy among patients of African ancestry.

**Partial Pentasomy of Proximal 15q: Clinical and Molecular Cytogenetic Characterization.** *H.E. Roberts<sup>1</sup>, B. Levy<sup>2</sup>, H.A. Creswick<sup>1</sup>, P.R. Papenhausen<sup>3</sup>*. 1) Children's Hospital of The King's Daughters, Norfolk, VA; 2) Mount Sinai-NYU Medical Center, New York, NY; 3) LabCorp of America, Research Triangle Park, NC.

Numerous studies of the proximal portion of chromosome 15q have been published because this region is often involved in structural rearrangements, and it also contains several imprinted genes. To date, partial pentasomy of proximal 15q has never been reported.

**Case Report:** The patient was born to a 29-year-old mother. The prenatal history was uncomplicated. A female was born at 40 weeks by a vaginal delivery without any complications. The infant was found to have significant developmental delay at 7 months of age. On physical examination at 13 months of age at a genetics evaluation, her head circumference was less than the 5th percentile. Epicanthal folds, flat nasal bridge, and a high arched palate were present. Three areas of patchy hypopigmentation were present on the neck, shoulders and chest. Five, small cafe-au-lait spots were present on the trunk and extremities.

**Molecular Cytogenetics:** Chromosome analysis revealed an abnormal karyotype, 48, XX, +2mar. FISH analysis identified both markers to be ring chromosomes derived from chromosome 15. Further analysis using a 15q11.2 SNRPN DNA probe showed a single SNRPN hybridization signal on the smaller of the two rings and two SNRPN hybridization signals on the larger ring chromosome. The mother's karyotype was normal. Uniparental disomy for chromosome 15 was not present. The rings were found to be maternal in origin. A comparative genomic hybridization analysis is in progress to better delineate the break points.

In the literature, patients with 4 copies of the Prader-Willi/Angelman syndrome (PWS/AS) region consistently have mental retardation and minor dysmorphic features. Since our patient has 5 copies of the PWS/AS region, it is predicted that her phenotype would be more severely affected than those with 4 or fewer. Numerous patients with 3 or 4 copies of apparently the same molecularly defined region of 15q11.2 allow gene dosage-phenotypic comparisons with our patient.

**Growth Failure in a child with 47,XXX and Angelman syndrome due to an imprinting error.** *J. Siegel-Bartelt<sup>1</sup>, J. Greenberg<sup>1</sup>, D.R. Rada<sup>1</sup>, E. Louie<sup>2</sup>, M. Jamehdor<sup>1</sup>.* 1) Kaiser-Permanente, Los Angeles, CA; 2) Northern California Kaiser, San Jose, CA.

The phenotype in Angelman syndrome (AS) has been reported to vary based on etiology. Here we report a child with sex chromosomal aneuploidy, growth failure and AS due to a putative imprinting error. This 2.5 year old girl was born at term following amniocentesis for maternal age which showed 47,XXX. Her birth weight was 2600 gm. At age 2.5 years, she was proportionate, but very petite: length 72.4cm; weight 7.8k; and OFC 42.5cm. Facies showed deeply set eyes and bilateral preauricular sinus. Hands and feet were very small for her size, and she was hypotonic. She was preverbal, but able to roll, and sit briefly if placed. Pincer grasp had not yet developed. She has had no seizures. Growth hormone therapy was instituted after 2 failed growth hormone stimulation tests. Chromosome analysis showed 47,XXX.ish15q11~q13(D15S10x2). Methylation specific PCR analysis revealed a single 100 bp band only, typical of Angelman syndrome. Uniparental disomy studies show biparental inheritance of chromosome 15.

Gillessen-Kaesbach et al (*EurJHumGenet* 7:638-44) report a series of patients presenting with obesity and hypotonia reminiscent of Prader-Willi syndrome (PWS), but who have methylation studies characteristic of AS. The small hands and feet, growth failure, and hypotonia in this child support this expanded Angelman phenotype. This is the first report of growth hormone deficiency in AS (though it is seen in PWS). Of note, the bilateral preauricular sinus in this child is unusual for both 47,XXX and PWS/AS. Clinicians should be alert to this broader spectrum of findings in Angelman syndrome, including growth failure.

The relationship of the sex chromosome aneuploidy and imprinting defect is not clear, and may be coincidental. However, it is interesting to speculate a possible potentiating effect of the extra X on the imprinting defect, especially to explain the more severe phenotype in this child compared to the group described by Gillessen-Kaesbach et al.

**X-linked chondrodysplasia punctata due to ARSE mutations expansion of the phenotype and genotype. T.**

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At least five types of chondrodysplasia punctata (CDP) have been described. Mutations in the ARSE gene which codes for arylsulfatase E have been identified as a cause of chondrodysplasia punctata CDPX1 and brachytelephalangi type chondrodysplasia punctata in 9 patients. The skeletal defects seen in ARSE deficiency are clinically similar to warfarin embryopathy and vitamin K deficient embryopathy. We describe two unrelated infant males who were born at 29 and 32 weeks gestational age with chondrodysplasia punctata. Both infants had hydrocephalus, Dandy Walker cysts and upper airway difficulties. One patient also had alobar holoprosencephaly, multiple ventriculoseptal defects, atrial septal defect, patent ductus arteriosus, bilateral hydronephrosis and died at 2 days age. Dandy - Walker cysts have been reported in patients with CDP due to vitamin K deficient embryopathy (Menger et al,1997) and warfarin embryopathy (Kaplan,1985). Other CNS malformations have been observed in warfarin embryopathy. The activities of arylsulfatase E in cultured fibroblast membranes were 0.02 and 0.01 nmoles /hr/mg membrane protein respectively (same day control 0.78 nmoles/hr/mg) using 4-MUS as substrate as previously described ( Franco et al 1995). All 11 exons of the ARSE gene were PCR amplified using primers placed in flanking introns. Sequencing of the exons demonstrated in one patient a single base deletion in exon 8 in the codon for amino acid 426 which resulted in the creation of a premature stop codon 431 in exon 9. The other patient had mutations in adjacent codons that resulted in R394P and W395G amino acid changes. The first mutation resulted in the loss of an Mps II restriction site that was present in 68 normal alleles. The second mutation introduced an Apa I restriction site that was absent in 32 normal alleles. These patients extend the clinical phenotype of arylsulfatase E deficiency to include premature birth, Dandy-Walker cysts, cardiac and other congenital malformations. ARSE deficiency represents another multiple congenital anomaly syndrome caused by a metabolic defect.

**Impaired glucose tolerance in Williams syndrome.** *B.R. Pober<sup>1</sup>, E. Wang<sup>1</sup>, K. Petersen<sup>3</sup>, L. Osborne<sup>4</sup>, S. Caprio<sup>2</sup>.* 1) Dept Genetics, Yale Univ Sch Medicine, New Haven, CT; 2) Dept Pediatrics, Yale Univ Sch Medicine, New Haven, CT; 3) Dept Internal Med, Yale Univ Sch Medicine, New Haven, CT; 4) Dept Medicine, Univ Toronto, Toronto, Canada.

Williams syndrome (WS) is a clinically complex disorder caused by a chromosome 7 microdeletion. Several endocrine problems have been previously identified; we now report impaired glucose tolerance as an additional medical complication that requires anticipatory guidance, monitoring and treatment.

We administered a standard oral glucose tolerance test to 16 WS adults (mean age of 37) and 4 WS teenagers (mean age of 14). Patients were classified as having normal glucose tolerance, impaired glucose tolerance (IGT), or "silent" diabetes according to established criteria (Diabetes Care.22:S1;1/99).

Among the adult subjects, 5/16 (31%) had silent diabetes while 7/16 (44%) had IGT. The diabetics tended to be obese (mean body mass index, BMI, =30), the patients with IGT were overweight (mean BMI=26), while those with normal glucose tolerance had normal BMIs. Older subjects were more likely to have IGT or diabetes. The mean fasting @ 120 minute glucose values for subjects with diabetes, IGT, and normal glucose tolerance were: 121 @ 239 mg/dl; 94 @ 162 mg/dl; and 86 @ 104 mg/dl, respectively. The mean fasting @ 120 minute insulin values for these groups were: 11 @ 44 uU/ml; 13 @ 68 uU/ml; and 17 @ 42 uU/ml. Only 1 subject had an elevated HemoglobinA1C. Among the 4 WS adolescents, 3 had IGT and 1 had normal glucose tolerance.

Our data show that 75% of subjects with WS have silent diabetes or IGT following oral glucose tolerance testing. The general population frequencies of these disorders are ~5% and ~15%. Elevated insulin levels in those with IGT suggest that insulin resistance plays a role in the pathogenesis of their diabetes. One of the genes commonly deleted in WS must confer vulnerability to diabetes. Syntaxin1A plays a role in exocytotic insulin release; further research will be needed to determine if hemizygoty for syntaxin1A contributes to the development of diabetes in patients with WS.

**Phenotype of Pigment Dispersion Syndrome (PDS) in Italian families.** *A.M. Bovell<sup>1</sup>, G. Calabrese<sup>2</sup>, G. Palka<sup>2</sup>, M. Ciafrè<sup>2</sup>, L. Mastropasqua<sup>2</sup>, D. Ciancaglini<sup>2</sup>, K.F. Damji<sup>1</sup>*. 1) Univ of Ottawa Eye Institute, Canada; 2) Università di Chieti, Italy.

**Purpose:** PDS affects up to 4% of the caucasian population and is a common cause of secondary open angle glaucoma (OAG). This study describes the phenotypic characteristics of Italian families with PDS. **Methods:** Probands were identified, through a glaucoma clinic, as individuals with both PDS and glaucoma. Probands and willing family members were examined in a standardized manner. Individuals were classified for PDS into affected (2 or more of: heavy trabecular meshwork(TM) pigmentation, iris transillumination defects(TID), Krukenberg (K) spindle), suspect (1 of the 3 characteristics listed above), and unaffected (no characteristics listed above). Each individual was also classified, independently, for OAG status as affected (2 or more of: glaucomatous optic nerve damage, intraocular pressure > 21 mm Hg, glaucomatous visual field defect), suspect (1 of the 3 characteristics listed above), and unaffected (no characteristics listed above). Data were entered into Excel and analysis performed. **Results:** 7 families with 65 individuals were examined. There were 18 PDS affecteds, 10 suspects, 35 unaffecteds, & 2 unknown. The average age was approximately 40 in all groups. The male:female ratio was 2:1 for affecteds and 1:1 for unaffecteds. The affecteds were more myopic (-2.8D vs. -1.6D) and had higher IOP's (23.9 mmHg vs. 17.8 mmHg) than the unaffecteds. On a scale of 0 to +4, the affecteds had more TM pigmentation than unaffecteds (3.3 vs. 0.8). 66.7% of affecteds had K spindles and 55.6% had TID. There was no evidence of K spindles or TID in the suspect or unaffected groups. The percentage of those with OAG in PDS affected, suspect and unaffected categories was 44.4%, 0% and 3.1%, respectively.

**Conclusion:** We have identified seven Italian families with PDS and glaucoma. These families will form the basis for future molecular studies in the hopes of identifying the gene(s) involved in this important disorder.

**Phenotypic features of families with angle closure glaucoma in Chongqing, China.** *K.F. Damji<sup>1</sup>, Y. Tu<sup>2</sup>, A.M. Bovell<sup>1</sup>, C. Yuan<sup>2</sup>, P. Li<sup>3</sup>, W.G. Hodge<sup>1</sup>, H. Peng<sup>3</sup>.* 1) University of Ottawa Eye Institute, Canada; 2) Chongqing Fourth People's Hospital Eye Institute, China; 3) Chongqing University of Medical Science, China.

**Purpose:** Angle closure glaucoma (ACG) is a leading cause of blindness in China. Although evidence of heritability exists, a molecular basis has not yet been identified. We describe the phenotypic characteristics of families with ACG from Chongqing, China. **Methods:** We identified probands with gonioscopic evidence of ACG. We examined families comprised of probands and at least one family member willing to participate. Individuals were classified into affected ( $\geq 180$  appositional or synechial closure = N3 or N4 Scheie), suspect ( $\geq 180$  closure Scheie N2 with iris bombe), or unaffected (wide open angle) categories. Exclusion criteria included any secondary cause of ACG, trauma, or pseudoexfoliation. Data were entered into Excel and analysis was performed.

**Results:** 16 families with 69 individuals were identified: 27 affecteds (A), 4 suspects (S), and 38 unaffecteds (U). Eight families had  $\geq 2$  affecteds. There was a preponderance of females in each category (M:F ratio for A=22:4, S=4:0, U=28:10). Affecteds had significant differences vs. unaffecteds ( $p=0.002$ ): they were older (A=59.7, U=36.0 years), had higher IOPs (A=33.5, U=17.9 mmHg), more hyperopia (A=1.04, U= -0.95 diopters), shallower central (A=1.64, U=2.42 mm), and peripheral (A=0.42, U=0.94 Van Herrick) anterior chamber depths, fewer angle structures visible by gonioscopy (A=3.35, U=0.23 Scheie method), thicker lenses (A=4.30, U=3.88 mm), and shorter axial lengths (A=21.67, U=22.68 mm).

**Conclusion:** We describe phenotypic characteristics of Chinese families with ACG. These families may form the basis for future molecular analysis of this important disorder.



**A new locus for Seckel syndrome on chromosome 18p11.31-q11.2.** *A.D. Borglum<sup>1</sup>, T. Balslev<sup>2</sup>, A. Haagerup<sup>1</sup>, N. Birkebæk<sup>2</sup>, H. Binderup<sup>1</sup>, T.A. Kruse<sup>3</sup>, J.M. Hertz<sup>4</sup>.* 1) Institute of Human Genetics, Aarhus University, Aarhus, Denmark; 2) Department of Paediatrics, Aarhus University Hospital, Denmark; 3) Department of Clinical Biochemistry and Genetics, KKA, Odense University Hospital, Denmark; 4) Department of Clinical Genetics, Aarhus University Hospital, Denmark.

Seckel syndrome (MIM 210600) is a rare autosomal recessive disorder with a heterogeneous appearance. Key features are growth retardation, microcephaly with mental retardation, and a characteristic bird-headed facial appearance. We have performed a genome-wide linkage scan in a consanguineous family of Iraqi descent. By homozygosity mapping a new locus for the syndrome was assigned to a 30 cM interval between markers D18S78 and D18S866 with a maximum multipoint lod score of 3.1, corresponding to a trans-centromeric region on chromosome 18p11.31-q11.2. This second locus for Seckel syndrome demonstrates genetic heterogeneity and brings us a step further towards molecular genetic delineation of this heterogeneous condition.

**Acalvaria: Case report of a rare malformation.** *L.H. Seaver, N.R. Cooley, Jr.* Greenwood Genetic Ctr, Greenwood, SC.

Acalvaria is a rare defect. The bones of the membranous neurocranium are absent or extremely hypoplastic and the bony defect is skin covered. The viscerocranium and chondrocranium are intact. There have been approximately 24 cases reported previously, although only 3 have adequate neuropathologic data and are unquestionably skin-covered. Underlying brain defects have been described, including holoprosencephaly (Harris 1993). All well documented cases have been sporadic. The ultrasonographic differential diagnosis includes acrania due to anencephaly or amniotic bands, severe microcephaly, and hypocalvaria due to osteogenesis imperfecta, hypophosphatasia, or ACE inhibitor fetopathy. We report autopsy data from another case of this rare malformation.

The mother was a 21-year-old G3P0Ab2. The pregnancy was complicated by positive MSAFP (3.56 MoM). Ultrasonogram revealed acrania with dilated cerebral ventricles, split cerebral hemispheres and increased size of the cisterna magna. Labor was induced at 21 weeks gestation. Autopsy revealed absence of the frontal and parietal bones. The anterior margin of the foramen magnum was ossified; the posterior margin was cleft and unossified. There was agenesis of the vertebral arches at C1-C2. The entire defect was covered with intact skin except the occipital region where there was a large area of aplasia cutis congenita. The brain revealed cleaved cerebral hemispheres with short blunted frontal lobes. The olfactory nerves were absent. The skull base was poorly differentiated. The corpus callosum was intact. The lateral ventricles were moderately dilated. Karyotype was normal 46,XX.

The etiology and pathogenesis of this defect is unknown. It may be due to failure of migration or differentiation of neural crest derived mesenchyme that forms the membranous bones of the skull. Mutations in the genes *MSX2* and *ALX4* have recently been identified as having a role in skull development with mutations found in families with parietal foramina/hereditary cranium bifidum (Wilkie 2000; Wuyts 2000; Mavrogiannis 2001). Some individuals with this condition also have occipital scalp defects.

**Vascular disruption-related birth defects in 22q11.2 deletion syndromes.** *K.J. Galenzoski<sup>1</sup>, C. Prasad<sup>1</sup>, A.J. Dawson<sup>1,2</sup>, A.E. Chudley<sup>1</sup>.* 1) Pediatrics and Child Health, Health Sciences Centre, Winnipeg, Manitoba, Canada; 2) Cytogenetics Laboratory, Health Sciences Centre, Winnipeg, Canada.

The 22q11.2 deletion syndrome has a variable phenotype involving craniofacial, cardiac and developmental features. The many anomalies characteristic of 22q11.2 deletion may be the result of a vascular disruption or a vascular developmental anomaly sequence. We describe two patients with deletions of 22q11.2: one with cutis aplasia and the other with gastroschisis, both suspected to be caused by vascular compromise. The first patient presented at birth with craniofacial dysmorphism including a cleft palate, dysplastic ears and micrognathia as well as an atrial septal defect and a right-sided aortic arch. He also had two circular lesions in his scalp, measuring two centimeters in diameter. Fluorescent-in-situ-hybridization analysis with D22S75 revealed a deletion on one chromosome 22, confirming the suspected diagnosis of a 22q11.2 deletion syndrome. The second patient presented antenatally with gastroschisis, and was found to have craniofacial dysmorphisms not typical for a 22q11.2 deletion syndrome. He had a cleft palate, an atrial septal defect and a bifid right thumb. FISH studies were positive for a 22q11.2 deletion. Vascular disruption has been implicated as a cause of aplasia cutis congenita (ACC) in patients with Adams-Oliver syndrome and ACC with terminal transverse limb defects. Aplasia cutis congenita is also seen in patients with teratogen exposure such as alcohol and cocaine, both of which are thought to cause vascular compromise. The gene Claudin-5/TMVCF (transmembrane protein deleted in Velo-cardio-facial syndrome) codes for a transmembrane protein that acts as a regulator of vascular permeability. It is located in the region of 22q11.2. Hemizygous expression of Claudin-5/TMVCF in individuals affected by 22q11.2 deletions may cause the secondary vascular disruption and resultant vascular anomalies in patients with 22q11.2 deletions. In addition, Claudin-5/TMVCF may be a potential candidate gene for familial forms of cutis aplasia and gastroschisis.

**A de novo 9q terminal deletion detected by subtelomere FISH analysis in a mildly affected child.** *M. He<sup>1</sup>, C. Lytle<sup>1</sup>, P. Challinor<sup>1</sup>, M. Sapeta<sup>1</sup>, B. Ravnar<sup>1</sup>, M. Irons<sup>2</sup>, A. Lamb<sup>1</sup>.* 1) FISH, Genzyme Genetics, Santa Fe, NM; 2) Boston Children's Hospital, Boston, MA.

Mental retardation is found in 3% of the general population. Of these, 40% of moderate to severe cases and 70% of the mild cases are unexplained. In a search for subtle rearrangements involving the ends of chromosomes, one large study (Knight et al., *Lancet* 354: 1676-1681, 1999) detected unbalanced rearrangements in 7.4% of the moderate-severe MR group and in only 0.5% of the mild MR group. We report on a 3-year-old male with normal chromosomes that was referred for subtelomere FISH analysis. His features include: moderate global delay, hypotonia, minor dysmorphic facies, hypertelorism, telecanthus, anteverted nares, borderline low set ears with folded helices. His head circumference was slightly less than the 25th centile, with his weight at the 50th centile, and height at the 50th to 75th centiles. Subtelomere FISH analysis (Totelvysion probes; Vysis, Inc) showed a 9q subtelomere deletion, with no other rearrangements observed. Follow-up studies using conventional FISH confirmed the deletion. Parental FISH studies were normal. Although the study cited above suggests a very low detection rate, subtelomere FISH analysis may yield useful information when all other testing is exhausted. As rearrangements are found in additional mildly affected individuals, it will be interesting to see if certain phenotypic features provide clues as to which of these individuals may warrant subtelomere FISH studies.

**Familial cryptogenic cirrhosis.** *J.L. Williams, M.S. Williams.* Gundersen Lutheran Medical Ctr, La Crosse, WI.

A family was identified with 6 individuals in 5 generations with liver disease. Medical records were available on 3 individuals (a mother and two daughters). All were diagnosed with cirrhosis. Extensive evaluation did not identify an etiology leading to categorization as cryptogenic cirrhosis. Pedigree suggests autosomal dominant (AD) inheritance, although X-linked inheritance cannot be ruled out. Age of onset for the confirmed cases was between 50-60 years. All three individuals had Type II diabetes. The family history was also positive for a variety of possible autoimmune disorders (pancytopenia, alopecia and vitiligo), although there was no evidence of autoantibodies directed against the liver. Iron studies and mutation analysis ruled out hemochromatosis. Alpha-1-antitrypsin levels were normal.

Cryptogenic cirrhosis accounts for 10% of all cases of cirrhosis. Familial cryptogenic cirrhosis has been reported rarely in the literature. Most of the families have not been as extensively investigated as this family therefore could represent other disorders. Recently some individuals with cryptogenic cirrhosis have been identified with dominant mutations in the keratin-8 and keratin-18 genes. Mouse mutants demonstrate AD inheritance of susceptibility to cirrhosis in some strains while other strains show a combination of cirrhosis with intestinal disease that resembles inflammatory bowel disease. It is not clear whether presence of the mutation is sufficient in and of itself to produce cirrhosis, or whether the mutation predisposes to abnormal hepatocyte response to toxins or stress. In transfected hepatocytes, mutations do not affect the organization of keratin filaments under basal conditions, but result in substantial abnormal reorganization of the filaments in response to a variety of stressors. Keratin mutation analysis in this family is being undertaken.

**A Gender Assessment Team: Experience with 183 patients over a period of 20 years.** *M.A. Parisi<sup>1,2</sup>, M.W. Burns<sup>1,2</sup>, R.E. Grady<sup>1,2</sup>, D. Gunther<sup>1,2</sup>, G.B. Kletter<sup>1,2</sup>, E. McCauley<sup>1,2</sup>, M.E. Mitchell<sup>1,2</sup>, C. Pihoker<sup>1,2</sup>, L.A. Ramsdell<sup>1</sup>, M.R. Soules<sup>2</sup>, R.A. Pagon<sup>1,2</sup>.* 1) Children's Hosp & Reg Med Ctr, Seattle, WA; 2) University of WA, Seattle, WA.

In 1981, the Gender Assessment Team was created at the University of Washington and Children's Hospital and Regional Medical Center to provide a multi-disciplinary approach to the diagnosis, medical and surgical treatment, and psychosocial evaluation of patients with ambiguous genitalia, intersex disorders, and other congenital anomalies of the genitalia. This group, consisting of specialists from medical genetics, cytogenetics, gynecology and the pediatric specialties of urology, endocrinology, and psychology, has meet bimonthly for 20 years. The findings of the 183 patients evaluated during this period are reviewed. Through a variety of laboratory and clinical tests, a determination of the chromosomal sex, gonadal sex, and phenotypic sex was made for each patient. This information was used in consultation with the family to reach decisions regarding management, including sex of rearing.

Categories of patients included infants with ambiguous or anomalous genitalia (128), of which 72 had Y chromosomal material and 56 had 46,XX karyotypes. 10 infants had intersex problems, in which the chromosomal sex did not match the phenotypic sex. 34 patients were referred in childhood or adolescence for intersex problems and anomalous genitalia. An additional 11 patients had ambiguous genitalia associated with a known multi-system genetic syndrome, such as Denys-Drash (3). Of specific diagnoses, the most common were: congenital adrenal hyperplasia (14%); clitoral and/or labial anomalies (9%); mixed gonadal dysgenesis (9%); hypogonadotropic hypogonadism with micropenis (8%); 46,XY SGA males with micropenis (7%), a previously recognized but underreported condition; and complete androgen insensitivity (7%). As a result of this experience, patients with 2 new syndromes have been identified: 1 46,XY female with agenesis of the corpus callosum and lissencephaly; and 3 males with micropenis, distinctive gonadotropin profile, microcephaly and learning delays.

**Is mosaicism at the origin of the extreme variability of 13q deletion phenotype?** *G. Andria, D. Melis, L. Perone, M.P. Sperandeo, A. Passariello, M. Sibilio, G. Sebastio.* Dept Pediatrics, Federico II Univ, Naples, Italy.

We observed a 13 month-old-female presenting facial dysmorphic features, skeletal anomalies (vertebral synostosis, multiple hemivertebrae, 13 ribs bilaterally), severe mental retardation and brain developmental anomalies (agenesis of the corpus callosum and Dandy-Walker variant). Eye fundoscopic examination revealed corioretinal yellowish lacunae in the right eye, similar to those seen in Aicardi syndrome. Karyotype analysis on peripheral lymphocytes was normal whereas del(13)(q12qter) was present in all mitoses from skin fibroblasts. Chromosome 13 painting excluded the translocation of the deleted segment. The mosaic pattern of the deletion was confirmed by molecular analysis of DNA microsatellites. Parents peripheral blood karyotypes were normal. Somatic mosaicism for del(13)(q12qter) has been reported in another patient only, presenting DK phocomelia phenotype which includes: radial ray defects, occipital encephalocoele and urogenital abnormalities. Large 13q deletions have been rarely observed whereas many patients with interstitial deletions have been reported. Considering all the clinical findings observed in both group of patients, the resulting phenotype might include: mental retardation, failure to thrive, developmental anomalies of brain, eye, heart and kidney, gastrointestinal system malformations and limb defect. No straight correlation between cytogenetic data and phenotype could be obtained. Surprisingly, the patient with the most extended deletion (13)(q12qter) was affected by a mild phenotype resembling the Moebius syndrome. The only two patients with a demonstrated mosaic pattern of the deletion, the one with the DK phocomelia phenotype and the present one, exhibit severe clinical pictures, only partially overlapping. We hypothesize that, beside to the extent and location of the deleted segment, the mosaicism for haploinsufficiency may underlie the wide variability of 13q- syndrome phenotype. Therefore, when possible, karyotype analysis of patients with 13q deletion seen in peripheral lymphocytes, should be extended to skin fibroblasts.

**Genotype-phenotype correlations in hypochondroplasia: progress report from an international collaborative project.** *A.S. Aylsworth<sup>1</sup>, G.A. Bellus<sup>2</sup>, S.H. Blanton<sup>3</sup>, T.E. Kelly<sup>3</sup> and The Genentech Hypochondroplasia Study Group.*  
1) Depts Pediatrics & Genetics, Univ North Carolina, Chapel Hill; 2) Dept Dermatology, Univ Colorado Health Sciences Center, Denver; 3) Dept Pediatrics, Univ Virginia, Charlottesville.

**BACKGROUND:** Hypochondroplasia is a common, autosomal dominant, genetically heterogeneous, skeletal dysplasia with variable clinical features that are similar to, but milder than those of achondroplasia. Molecular studies have shown that 50-70% of cases are caused by mutations that result in an Asn®Lys substitution in codon 540 of fibroblast growth factor receptor 3 (FGFR3). We are assembling a large cohort of patients with clinical diagnoses of hypochondroplasia to determine genotype-phenotype correlations and to search for mutations other than the common ones. The standardized study data set includes clinical findings, radiographs, and FGFR3 mutation analysis. **RESULTS:** We now have some data on approximately 250 patients, but complete datasets on only one third of these. Molecular testing on 236 patients shows that 58% have FGFR3 mutations (N540K or K650N/Q). There is little difference in height, arm span, or head circumference between adults with and without FGFR3 mutations. Both groups have a higher than expected incidence of neurological problems (»50%). Data regarding radiological findings and growth curves for FGFR3(+) and FGFR3(-) patients will be presented. **FUTURE PLANS and ACKNOWLEDGEMENTS:** It is becoming more difficult to organize and participate in multicenter studies because IRB requirements have become much more stringent during the past two years. We greatly appreciate those collaborating members of the Study Group who have submitted complete datasets. Their names and affiliations are listed on the poster. We will be working over the next year to obtain missing data, add new patients to the database, help submit local IRB protocols where necessary, and identify candidates for the human growth hormone trial arm of the study. Web access to protocols and consent forms should help study group collaborators add data or new study subjects more easily, and construct local IRB consent forms when necessary.



**Angelman syndrome with OCA2 due to an intragenic deletion of the P gene.** *C.P. Koiffmann<sup>1</sup>, C. Fridman<sup>1</sup>, N. Hosomi<sup>2</sup>, M.C. Varela<sup>1</sup>, A.H. Souza<sup>3</sup>, K. Fukai<sup>2</sup>.* 1) Dept of Biology, Univ of Sao Paulo, Sao Paulo, Brazil; 2) Dept of Dermatology Osaka City University Graduate School of Medicine, Osaka, Japan; 3) Instituto Materno-Infantil de Pernambuco, Neurologia Infantil, PE, Brazil.

Angelman syndrome (AS) is characterized by mental retardation, speech impairment, ataxia and happy disposition with frequent smiling. AS results from the loss of expression of a maternal imprinted gene in the 15q11q13 region, due to deletion (70%), paternal uniparental disomy (5%), IC mutation (1-2%) or UBE3A mutation (10%); in the remaining cases the cause is unknown. Deletion AS patients may exhibit hypopigmentation of skin, eye and hair correlating with deletion of one copy of the P gene mapped in the distal part of PWS/AS region. AS patients may occasionally present features of OCA2, the most prevalent type of albinism worldwide, due to a mutated P gene in the remaining paternal chromosome 15. Our patient presented albinism (his parents were of African ancestry), developmental delay, severe mental retardation, absence of speech, outbursts of laughter, microcephaly, ataxia, hyperactivity, seizures. The study of SNRPN gene showed the methylation pattern of AS; microsatellite analysis of chromosome 15 disclosed deletion with breakpoints in BP2 and BP3. All of the 25 exons and flanking introns of the P gene of the patient and his parents were investigated. The father carries the deleted allele and the patient is hemizygous for the deleted exon 7 of the P gene paternally inherited. The patient manifests OCA2 associated with AS due to the loss of the maternal chromosome 15 with the normal P allele, since the paternal breakpoints include the P gene. The intragenic deletion of the paternal P gene results in a frameshift mutation predicting a non-functional protein reflecting in the patient's white skin, gold hair and no retinal pigmentation. As various degrees of hypopigmentation are associated with PWS/AS deletion patients, the study of the P gene present in a hemizygous state could contribute to the understanding of its effect on human pigmentation during development and to disclose the presence of modifier pigmentation gene(s) in the PWS/AS region. Supported by: FAPESP, CNPq.

**Molecular Diagnosis of Smith-Lemli-Opitz Syndrome (SLOS) in a Fetus with Renal Agenesis. M.J.M.**

*Nowaczyk*<sup>1,2,3</sup>, *S.A. Farrell*<sup>2,4</sup>, *W.S. Sirkin*<sup>5</sup>, *B. Eng*<sup>3</sup>, *J.S. Wray*<sup>1,2,3</sup>. 1) Dept. of Pathology and Molecular Medicine, McMaster University, Hamilton, Canada; 2) Dept. of Pediatrics, McMaster University, Hamilton, Canada; 3) Hamilton Regional Laboratory Medicine Program, Hamilton, Canada; 4) Division of Genetics, The Credit Valley Hospital, Mississauga, Canada; 5) Dept. of Laboratory Medicine, North York General Hospital, North York; Canada.

We report on a fetus with oligohydramnios and renal agenesis detected sonographically in the second trimester of pregnancy, in whom the diagnosis of SLOS was made based on molecular testing of *DHCR7* in the parents. Amniocentesis showed a normal male karyotype, 46,XY. The pregnancy was stopped because of the severity of fetal anomalies. The parents were not consanguineous; their ethnicities were Tatar-Tatar and Russian-Ashkenazi.

The fetus had facial features of Potter oligohydramnios sequence; there was a cleft posterior palate, and bilateral postaxial polydactyly of the toes with syndactyly of the fifth and sixth toes. External genitalia were ambiguous; internal genitalia were male. The right kidney was absent; the left was markedly hypoplastic as were both ureters. There were multiple accessory spleens, as well as a spleen in the usual location. There was bilateral pulmonary hypoplasia with a bilobed right lung and unilobar left lung; an atrioventricular septal defect; and, in the brain, agenesis of the corpus callosum and a Dandy-Walker malformation. In the hypoplastic kidney there was incomplete nephrogenesis with markedly decreased glomerular generations. Clinical severity score was 72.

As there was no amniotic fluid or cultured cells available for biochemical testing, the parents underwent molecular analysis of *DHCR7*: the mother's genotype was IVS8-1G>C/+, the father's W151X/+. Both of these *DHCR7* mutations are null mutations which are associated with a severe phenotype.

This report demonstrates the utility of carrier molecular testing for SLOS in situations where samples are not available from the proband. This is also a first report of confirmed SLOS in fetus of this ethnicity (Ashkenazi and Tatar).

**Defining the deletion breakpoints in WS patients using somatic cell hybrids.** *K.A. Metcalfe<sup>1</sup>, M.J. Carette<sup>1</sup>, D. Donnai<sup>1</sup>, A. Karmiloff-Smith<sup>2</sup>, A.P. Read<sup>1</sup>, M. Tassabehji<sup>1</sup>.* 1) University Department of Medical Genetics and Regional Genetics Service, St Mary's Hospital, Manchester, UK. , St Mary's Hosp, Manchester, England; 2) Neurocognitive Development Unit, Institute of Child Health, 30 Guilford Street, London, U.K.

Williams-Beuren syndrome (WS) is a developmental disorder caused by a hemizygous microdeletion of ~1.5Mb at chromosomal location 7q11.23. Homologous recombination between flanking repeats accounts for high incidence of de novo deletions. Up to 20 genes have been identified within the WS critical region, many of which are predicted to encode transcription factors, some have roles inferred from motif homologues, and some have no known function. Hemizyosity for ELN causes SVAS and currently there is no evidence implicating any of the other genes in the aetiology of the syndrome. Our studies of rare patients ascertained through SVAS who have partial deletions but not WS, suggest that the telomeric end of the common deletion is important for determining the WS phenotype. Mapping the exact deletion breakpoints is difficult because they lie within the repeated regions. In order to define the deletion breakpoints more precisely we have made somatic cell hybrids from 30 WS patients and designed a series of specific PCR primers using SNP technology to identify the centromeric and telomeric breakpoints. There appear to be two main breakpoints at the centromeric end and at least three at the telomeric end which has allowed us to group the patients according to their genotype for further detailed phenotype analysis. This should aid future genotype-phenotype correlations.

**Craniofacial anthropometry in cleido-cranial dysplasia using three dimensional surface laser imaging studies.** *B. Lee, S. Carter, CA. Bacino.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Cleidocranial dysplasia (CCD) is a skeletal disorder that results from loss of function of the RUNX2/CBFA1 gene. This gene is a transcriptional determinant of osteoblast cell fate. Patients with CCD present with classical dysmorphic craniofacial features including: prominent forehead, hypertelorism, flat nasal bridge, receding midface, and prognathism. Multiple studies have addressed the craniofacial changes in CCD mainly by cephalometric measurements and by CT scans with 3D reconstructions. A number of abnormalities have been observed including delayed ossification of calvaria, abnormalities of the cranial base, delayed ossification of the cranial base bones, poorly developed maxillary and zygomatic bones, absent or hypoplastic nasal bones, and mandibular anomalies. While these approaches can demonstrate the bony abnormalities, they do not evaluate the total soft tissue contribution to the dysmorphic facies. The use of laser surface scans potentially offers a different approach to the study and diagnosis of craniofacial abnormalities by enabling linear and volumetric measurements. This technique allows the analysis of surface texture with the inclusion of soft tissues in the evaluation of morphological changes in CCD patients as well as in other craniofacial disorders. We have studied six individuals with CCD with known CBFA1 mutations by 3-D laser surface imaging studies to address this issue. Six age matched control individuals were also studied for comparison purposes. Preliminary results indicate that CCD patients have characteristic craniofacial anomalies, in particular abnormalities of the midface which are evident on both linear and volumetric measures. Our final goal is the translation of mutation analysis into the clinical phenotype. Genotype-phenotype correlations will help to elucidate variable clinical expressivity of mutations that may result in haploinsufficiency vs. hypomorphic mutations. Ultimately, the generation of volumetric measures of soft tissues of the different facial sections in age matched-control populations will enable diagnostic quantitative assessment of dysmorphic features in this and other craniofacial disorders.

**Nonsense and frameshift mutations in *ZFHX1B*, encoding Smad interacting protein-1, cause a complex developmental disorder with a variety of neurocristopathies.** *K. Yamada*<sup>1</sup>, *Y. Yamada*<sup>1</sup>, *N. Nomura*<sup>1</sup>, *K. Miura*<sup>3</sup>, *R. Wakako*<sup>4</sup>, *C. Hayakawa*<sup>5</sup>, *A. Matsumoto*<sup>7</sup>, *T. Kumagai*<sup>5</sup>, *I. Yoshimura*<sup>4</sup>, *S. Miyazaki*<sup>7</sup>, *K. Kato*<sup>2</sup>, *S. Sonta*<sup>1</sup>, *H. Ono*<sup>7</sup>, *T. Yamanaka*<sup>5</sup>, *N. Nagaya*<sup>6</sup>, *N. Wakamatsu*<sup>1</sup>. 1) Dept Genet; 2) Dept Biochem, Inst Developmental Res; 3) Dept Pediatr Neurol; 4) Dept Psychiatr; 5) Dept Pediatr; 6) Dept Pediatr Surg Central Hosp; 7) Resid Fac Child Mentl Physic Disabil, Aichi Human Service Center, Kasugai, Aichi, Japan.

Mutations in *ZFHX1B*, encoding Smad interacting protein-1, have been recently described to cause a form of Hirschsprung disease (HSCR). To investigate the breadth of the clinical variations of *ZFHX1B* deficiency, we have analyzed *ZFHX1B* from five patients presenting with mental retardation, delayed motor development, epilepsy, microcephaly and mild facial dysmorphism. These clinical features are quite similar to those of reported earlier except that they are not typical HSCR cases. The results showed that a previously reported R695X mutation could be identified in three patients. Moreover, a 2-bp insertion (254fs262) and a single bp deletion (91fs107X) were also newly found in two cases. All mutations occurring in one allele were de novo events. These data demonstrate that *ZFHX1B* deficiency is an autosomal dominant complex developmental disorder and that patients carrying functional null mutations of *ZFHX1B* present delayed physical and mental development and epilepsy with a wide spectrum of clinically heterogeneous neurocristopathies at the cephalic, cardiac and vagal levels.

**A novel syndrome of elevated serum IgE, recurrent infections, vasculitis, and neurocognitive impairment with myoclonus.** *B.N. Hay<sup>1</sup>, J. Davis<sup>1</sup>, D. Darnell<sup>2</sup>, B. Karp<sup>3</sup>, J.E. Martin<sup>4</sup>, B.I. Solomon<sup>5</sup>, M.L. Turner<sup>6</sup>, J.M. Puck<sup>1</sup>, S.M. Holland<sup>7</sup>.* 1) Genetics and Mol. Biol. Branch, NHGRI, NIH, Bethesda, MD; 2) Clinical Center, NIH, Bethesda, MD; 3) NINDS, NIH, Bethesda, MD; 4) Dept. of Internal Medicine, Johns Hopkins Bayview Medical Center, Baltimore, MD; 5) Rehab. Med., Clinical Center, NIH, Bethesda, MD; 6) National Cancer Institute, NIH, Bethesda, MD; 7) Lab of Host Defenses, NIAID, NIH, Bethesda, MD.

We present a family with 5 of 6 siblings (including male twins) affected with a complex immune disorder resembling Hyper-IgE syndrome (HIES), which typically manifests as recurrent boils, cyst-forming pulmonary infections, extremely elevated serum IgE levels and non-immunologic connective tissue abnormalities. The 3 affected male and 2 affected female children had severe eczema starting at 9 months of age, recurrent infections, episodes of post-infectious Stevens-Johnson syndrome in early childhood, and IgE levels ranging from 9,400 to 34,500 IU/ml. Three children have had chronic sinusitis or otitis, cutaneous vasculitis, and recurrent bacterial pneumonias leading to bronchiectasis and pulmonary compromise. Although no one in this family had documented pneumatoceles characteristic of HIES, one child died of respiratory failure at 27 years of age. One surviving child has severe destruction of pulmonary parenchyma due to recurrent infections with streptococci and staphylococci. In addition, one of the twins has had recurrent parotitis requiring parenteral antibiotics. Of the non-immunologic features of HIES, mild to moderate scoliosis was found in 3 of the children, but retained primary teeth and hyper-extensible joints were not present. Neurologic features, not typically part of HIES, were documented in all of the affected family members including oral motor deficits and dysarthria, low average IQ (75-80), increased deep tendon reflexes and essential myoclonus. The nonconsanguineous parents and one sibling had no significant findings, suggesting an autosomal recessive mode of inheritance. This family exhibits a unique constellation of immunologic and neurologic abnormalities that to our knowledge has not previously been reported.

**SHOX gene deletion in a male patient without short stature and Madelung deformity.** *f. faravelli<sup>1</sup>, g. camera<sup>2</sup>, m. pierluigi<sup>1</sup>, m. baldi<sup>1</sup>, f. dagna<sup>1</sup>.* 1) Laboratory of Human Genetics, Ospedale Galliera, Genova, Italy; 2) Pediatric Department, Ospedale San Paolo, Savona, Italy.

Deletions and mutations in the SHOX gene are responsible for Leri-Weill disease (LWD), a skeletal dysplasia characterised by mesomelic shortening of limbs and Madelung deformity. Recently, the same gene has been proposed to be responsible for as many as 2% of cases of idiopathic short stature. We report on a patient who has been ascertained because he is member of a family where Leri-Weill disease is segregating. His affected mother and sister presented with the full clinical picture of the disorder (short stature and Madelung deformity). The propositus asked for genetic counselling concerning his reproductive risk for LWD. No physical anomalies were detected on examination and height was 1.74 m (mother has LWD AND is 1.45 m height, the father is 158 cm). X Rays of the upper limb was performed and excluded Madelung deformity although a minor anomaly was detected and described as ulna minus. On the basis of this finding, both FISH and molecular analysis were performed on affected members of the family and the propositus and revealed a SHOX gene deletion in the affected mother, affected sister and, surprisingly, in the propositus. This observation confirms the extreme phenotypic variability which is associated with LWD, providing a further example of difficult tasks faced in genetic counselling. Moreover, the lack of short stature in our propositus further complicates the discussion about the role of this gene as a major determinant in the pathogenesis of short stature, both syndromic and idiopathic.

**Lacunar skull and limb defect with 22q11 microdeletion: novel features of velo-cario-facial syndrome? S.E.**

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We describe a male patient showing at birth lacunar skull, cleft palate, thoracolumbar kyphoscoliosis, congenital cardiopathy, intercalar defect of limb at cost of left femur and retardation of neuropsychomotor development. As obstetric antecedents he presented intrauterine low motion and retardation of development. At the age of 15 years 3 months the patient became dead due to cardiac complications secondary to cardiac arrhythmia. The cariotype of peripheral blood lymphocytes was normal, 46,XY in 11 cells. By virtue of facial likeness with Velo-Cardio-Facial Syndrome (VCFS) a molecular analysis was performed and revealed a 22q11.2 interstitial deletion. It is discussed in this article the differential diagnosis between VCFS and Adams-Oliver Syndrome (AOS) among others, based on clinical features showed by the patient because up to the moment there are no studies in molecular biology relative to AOS. The authors conclude that this is the first described case of lacunar skull and intercalar defect of limbs with 22q11.2 microdeletion.



**Etiological Classification of Congenital Limb Anomalies.** *S.M. Tayel<sup>1</sup>, S.A. Al-Awadi<sup>1</sup>, L. Bastaki<sup>1</sup>, K.K. Naguib<sup>1</sup>, S.J. Abulhasan<sup>1</sup>, F.M. Mustafa<sup>1</sup>, S. Gouda<sup>1</sup>, N. Al-Naqeeb<sup>2</sup>.* 1) Kuwait Medical Genetics Centre, Maternity Hospital, Kuwait; 2) Neonatology Department, Adan Hospital\*, Kuwait.

Limb anomalies rank behind congenital heart disease as the most common birth defects in infants. More than 50 classifications for limb anomalies based on description of the morphology and osseous anatomy are drafted over the past 150 years. The present study aims at providing a concise summary for the most common congenital limb anomalies based on its etiology using a recent etiological classification first presented in 1996. Seventy cases with anomalies of the upper and/ or lower limb(s) were ascertained through clinical examination, chromosomal analysis, skeletal surveys and other relative investigations. Fetal causes of limb anomalies presented 55.8% of the cases in the form of 9 cases (12.9%) with chromosomal aberrations (trisomies 13, 18, 21, 13q duplication, and 22q deletion) and 30 cases (42.9%) with single gene disorders. Environmental etiology of limb anomalies was diagnosed in 11 cases (15.7%) in the form of amniotic band disruption, monozygotic twin with abnormal circulation, vascular disruption (Poland sequence, sirenomelia, and general vascular disruption), and an infant of diabetic mother. Twenty cases (28.5%) had limb anomalies as part of a sporadic syndrome of unknown etiology. Etiological classification of limb anomalies adopted in the present work looks valuable for understanding the mechanisms involved in limb anomalies and appears crucial for its prevention through genetic counseling which provides ways for prenatal detection and recurrence risk estimation.

**Tuberous sclerosis associated with pulmonary vascular lesions, emphysema and Klippel-Trenaunay-Weber syndrome.** *D.K. Grange, J.S. Kemp, G.K. Singh, T.R. Weber, A.Z. Miklos, D.E. deMello, C. Sotelo-Avila.* Departments of Pediatrics, Surgery and Pathology, Saint Louis University School of Medicine, St. Louis, MO.

We report a patient with tuberous sclerosis (TS) associated with pulmonary vascular abnormalities and emphysema, as well as Klippel-Trenaunay-Weber syndrome (KTW) of the upper extremity. TS is associated with hamartomatous lesions of brain, kidney, skin and, rarely, lung. KTW is associated with vascular malformations and asymmetric limb overgrowth and may have pulmonary involvement. A 2-year-old boy was born with an enlarged right upper limb with overlying cutaneous vascular lesions. MRI/MRA scan showed soft tissue hypertrophy of the limb and vascular abnormalities involving the arm, forearm and thenar eminence. In addition, multiple cardiac rhabdomyomata, hamartomas in the brain and shagreen patches were identified. He has had infantile spasms, complex seizures and developmental delays. At 17 months, he suddenly developed hemoptysis. Angiogram demonstrated a pulmonary artery malformation in the right lower lobe, requiring a lobectomy. Pathologic examination revealed large tortuous hilar bronchial arteries containing thrombi, with adjacent hemorrhage into the bronchial lumen, consistent with hemorrhage from a bronchial vascular malformation. Large pulmonary arteries had medial muscular hypertrophy and altered elastic fibers. There was also patchy emphysema. Abnormal arteries were seen in the periphery of the lobe within the emphysematous area, and there were increased numbers of dilated capillaries in the visceral pleura. Localized gigantism has been reported rarely in TS, including isolated macrodactyly and segmental hemihypertrophy. The association of TS and KTW has been previously described (*J Neuro Neurosurg Psych* 38:500-504,1975). Lymphangioliomyomatosis (LAM) and cystic disease are known pulmonary complications in TS, usually found in adult females, however, a male with KTW and pulmonary lymphatic involvement similar to LAM has been reported (*Chest*, 102:1274-77,1992), suggesting there may be overlap in the pulmonary manifestations of TS and KTW. Mutational analysis of TSC genes may provide insight into the pathogenesis of the defects seen in this and other patients.

**A Novel PTEN missense mutation in a patient with macrocephaly, ventricular dilatation and features of VATER association.** *W. Reardon<sup>1</sup>, X-P. Zhou<sup>2</sup>, C. Eng<sup>2</sup>.* 1) Dept Clin Gen, Ctr Medical Gen, Our Lady's Hosp Sick Children, Dublin, Ireland; 2) Human Cancer Genetics Program, The Ohio State University, Columbus, OH.

Initially described in the autosomal dominant condition of Cowden syndrome, germline mutations at the PTEN locus on 10q22-23 have now been extended to a wider range of phenotypes. Pathogenic mutations have additionally been described in Bannayan-Riley-Ruvalcaba syndrome and patients with Proteus and clinically overlapping syndromes. In contrast a molecular basis to VATER association remains undetermined in almost all cases of that condition. We report a novel missense mutation H61D at the PTEN locus in a patient with macrocephaly associated with significant ventricular dilatation on cranial imaging but whose additional phenotypic features of radial hypoplasia, bilateral thumb malformations and tracheo-esophageal fistula significantly overlap with the VATER phenotype. The mutation has arisen de novo in the patient, parental analyses being normal. Previous clinical experience of patients with VATER association and macrocephaly/hydrocephalus has highlighted this phenotype as a possible clinical presentation of chromosome breakage disorders. Our observation, in a patient with normal chromosomes, no increased sister chromatid exchange and a normal response to alkylating agents extends the range of phenotypes associated with PTEN mutation and offers clinicians a new investigative approach in patients with macrocephaly/hydrocephalus and VATER association.

**Phenotypic variation in individuals with or without cleft palate and their unaffected family members. K. Neiswanger, S.S. Petiprin, K.M. Bardi, M.D. Ford, A.S. Sauder, R.F. Giles, S.M. Weinberg, E.S. Carter, M.E. Cooper, M.L. Marazita.** School of Dental Medicine, University of Pittsburgh, PA.

Non-syndromic cleft lip with or without cleft palate (CL/P) is a common birth defect of unknown etiology that involves genetic and environmental factors. The Pittsburgh Oral-Facial Cleft Study is collecting a large series of multiplex CL/P families for linkage and segregation analyses. In addition to obtaining a blood sample, all family members--along with controls from families with no history of clefts, are evaluated for a number of sub-clinical developmental and laterality phenotypes that may reflect a genetic predisposition to clefting. These include a speech evaluation, a series of anthropological traits, and laterality measures such as handedness and dermatoglyphics. Pilot data from 66 individuals in 18 families have been collected. Results of the speech assessment suggest that velopharyngeal competence (measured by the University of Pittsburgh Weighted Values for Speech Symptoms Associated with Velopharyngeal Incompetence) does not differ between affected and unaffected family members ( $p = 0.19$ ). Dermatoglyphics include total ridge count (TRC) and fingerprint pattern frequencies, neither of which differ between CL/P individuals and unaffected relatives (mean TRC: 148.4 vs 134.9,  $p = 0.8$ ; pattern frequencies:  $p = 0.96$ ). Among the thirteen anthropological traits with sufficient data, only the presence of an elongated second toe shows a significant increase in CL/P individuals (60% vs 27%,  $p = 0.02$ )--a likely artifact of multiple testing. Handedness data (assessed by a modification of the Edinburgh Handedness Inventory) suggest that there may be more non-righthandedness among affected individuals than unaffected family members ( $p = 0.03$  or  $0.08$ , depending on the definition of non-righthandedness). Except for handedness, all phenotypic frequencies are similar in CL/P individuals and their unaffected relatives, suggesting that common genetic factors underlie these traits for all family members. Thus, these sub-clinical traits may be useful for family studies of CL/P and for refining recurrence risk estimates. Supported by NIH grant# DE13076.

Program Nr: 719 from the 2001 ASHG Annual Meeting

**Natural history, and genotype/phenotype correlation in a large cohort of Italian RTT patients with MECP2 mutations.** *M.L. Giovannucci Uzielli, L. Giunti, E. Lapi, U. Ricci, S. Guarducci, A. Cecconi, I. Sani, A. Elena.* Genetics and Molecular Medicine University of Florence Department of Paediatrics (Italy).

The collection of a large number of RTT patients with identified MECP2 mutations in the coding regions, included in the Register of our Center, allows us to get some consistent data concerning the natural history and clinical spectrum of the disorder. These data are especially useful for a genotype/phenotype correlation within the single groups of patients with the same MECP2 mutation. We identified 85/105 mutations (78%): T158M and R270X representing the most consistent types of mutations. The reconstruction of the natural history was facilitated, in this study, by the long period of follow-up, about 17 years, carried out in a large, and more and increasingly significant number of RTT patients. We have a special RTT Programme established in collaboration with the Italian Rett Syndrome Families Association (AIR). Other data are periodically collected by using special information forms, sent to the families. No consistent correlations were so far observed, neither for the genotype/phenotype correlation, nor for the patterns of X-inactivation. The study is in progress and we are also comparing our data with these collected by other groups of scientists involved in Rett Syndrome studies.

Program Nr: 720 from the 2001 ASHG Annual Meeting

**MECP2 mutation analysis and genotype/phenotype correlation in 26 Dutch Rett Syndrome patients.** *J. Herbergs, E. Smeets, U. Moog, D. Tserpelis, H. Smeets.* Genetics and Cell Biology, Maastricht University, Maastricht, Netherlands.

Rett syndrome is an X linked neurodevelopmental disorder that almost exclusively affects girls. The prevalence of the disease is estimated at 1/10.000 to 1/15.000. After an apparently normal prenatal and postnatal development a developmental stagnation appears, followed by regression of mental and motor abilities. In our lab the MECP gene was screened in 26 patients with classical and atypical RETT syndrome. In 7 patients (27%) a pathological mutation was detected which was not found in one of the parents. In 4 out of 7 patients the R306C mutation was detected, which is a mutation in the transcription repression domain. Most of the patients were adult women and in those patients a possible relationship between clinical outcome and DNA mutation type was investigated, especially for the R306C mutation. No clear correlation was detected between the detected mutations and the severity of the phenotype. More predictive for the clinical outcome appear to be the severity of the motor impairment, the progress of the corresponding neurological signs, and the age at onset of stagnation.

**Severe penicillamine embryopathy in an infant born to a woman with Wilson Disease.** *R.A. Pinter<sup>1</sup>, W.A. Hogge<sup>1</sup>, E. McPherson<sup>1, 2</sup>*. 1) Dept Genetics, Magee-Womens Hosp, Pittsburgh, PA; 2) Dept Pediatrics, Childrens Hosp Pittsburgh.

A 2190 gram male was born at 36 weeks to a 35 year-old G2P2 woman who took D-penicillamine (DP) 1g/d for Wilson disease throughout the first half of her pregnancy. The dose was reduced when a fetal ultrasound showed contractures and callosal agenesis. Maternal copper and zinc levels were not measured. The infant was born with severe cutis laxa, joint contractures, severe micrognathia, and callosal agenesis/colpocephaly on MRI. Amniocentesis karyotype was 46XY. His older half-sibling, also born to the mother while taking DP, has agenesis of the corpus callosum, but is otherwise normal. The proband's post-natal course was complicated by a tracheostomy due to micrognathia, mandibular distraction surgery, and orthopedic casting. At 6 mo follow-up, his cutis laxa and his joint contractures had improved, but he had profound developmental delay and apparent cortical blindness. Embryopathy is a rare complication of chronic use of DP during pregnancy. Roubenoff et al. in 1988 reported congenital anomalies in only 4/93 DP exposed infants. Arthrogryposis and CNS anomalies are documented in rats receiving DP prenatally, but only mild contractures are reported in humans. Two cases of embryopathy have been reported in offspring of mothers being treated with DP for Wilson Disease, and three born to mothers using DP for other purposes (collagen-vascular disease or cystinuria). Only two survived, both born to mothers with Wilson disease. All 5 were born with cutis laxa, thought to be secondary to copper and/or zinc deficiency, or as a result of direct toxic effect of DP on collagen and elastin crosslinks. In the surviving infants, the cutis laxa improved over several months and by age two months wound healing was normal. Neither had joint problems and both were neurologically normal. In summary, this is the most severe case of penicillamine embryopathy reported for an infant born to a woman with Wilson disease. Measurement of copper and zinc levels during pregnancy may have been helpful in predicting embryopathy and is suggested in other pregnant patients taking DP.

**Clinical and genetic analysis of Primary Open Angle Glaucoma (POAG).** *A. Renieri<sup>1</sup>, P. Frezzotti<sup>2</sup>, B. Palumbo<sup>1</sup>, R. Ciappetta<sup>2</sup>, M.G. Bucci<sup>3</sup>, S. Gandolfi<sup>4</sup>, M.T. Rolle<sup>5</sup>, N. Orzalesi<sup>6</sup>, I. Zucca<sup>7</sup>, L. Tomazzoli<sup>8</sup>, R. Frezzotti<sup>2</sup>.* 1) Dept. Mol. Biol., Medical Genetics, University of Siena, Italy; 2) Dept. Ophthalmol. Science, University of Siena, Italy; 3) Dept. Ophthalmol. Science, University of Rome Tor Vergata, Italy; 4) Dept. Ophthalmol. Science, University of Parma, Italy; 5) Dept. Physiopathol., Sez. Ophthalmol., University of Torino, Italy; 6) Dept. Ophthalmol. Science, University of Milano, Italy; 7) Dept. Ophthalmol. Science, University of Cagliari, Italy; 8) Dept. Ophthalmol. Science, University of Verona, Italy.

We have collected 52 families in which more than one individual was affected with glaucoma (G). Four families had six to eight affected members. The remaining families were small and the DNA of proband and two healthy relatives were only available, with few exceptions. All families had an apparent autosomal dominant inheritance on the basis of formal genetic analysis.

An accurate clinical examination of all families revealed some degree of variability of phenotype. In one family there was the simultaneous presence of Primary Open Angle G. -POAG-(4 individuals), Normal Pressure G. -NPG-(2 individuals) and Chronic Angle Closure G. -CACG-(1 individual). NPG and PAOG also coexisted in a smaller family. In another family CACG (1 individual) and Acute Angle Closure G (1 individual) coexisted with POAG (4 individuals). In one family one patient had POAG and the other Intermittent Angle Closure G. In one family with two affected members the Pseudo Exfoliation variety of Primary Open Angle Glaucoma segregated. In nine families one or two individuals among three affected members fulfilled the criteria for Juvenile Open Angle G.

Molecular analysis of Myocilin gene performed by SSCP and direct sequencing on proband's DNA of each family did not reveal pathogenic alterations. Our results confirm that mutations in Myocilin gene are rare in autosomal dominant POAG. Moreover, our analysis suggests a common molecular basis for entities distantly related on clinical ground only.



**Compound heterozygosity for FGFR3 gene mutations in a family with hypochondroplasia.** *A. Winterpacht<sup>1</sup>, C. Steglich<sup>1</sup>, K. Salzgeber<sup>2</sup>, D. Glaeser<sup>3</sup>.* 1) Inst Human Genetics, Univ Hamburg, Hamburg, Germany; 2) Childrens Hospital, Bismarckstr. 23, Memmingen, Germany; 3) Gregor Mendel Laboratorien (GML), Wegenerstr. 15, Neu-Ulm, Germany.

Hypochondroplasia (HCH) (MIM 146000) is an autosomal dominant skeletal dysplasia which is caused by mutations in the fibroblast growth factor receptor 3 (FGFR3)- gene and which is allelic with other dwarfism and craniosynostosis syndromes, like Achondroplasia, Thanatophoric dysplasia and Muenke syndrome. In HCH patients five different mutations in the FGFR3 gene have been identified, so far: a N540K exchange (caused by two different base substitutions) which is present in about 40-60% of the patients, a N540T and N540S as well as an I538V and N328I exchange which have been reported in rare cases. Most of the mutations affect the first portion of the split intracellular tyrosine kinase domain of the receptor and, like the other FGFR3 mutations, cause a ligand independent activation of the receptor. Here, we report the case of an 11 year old girl with a rather severe form of HCH (originally diagnosed as mild Achondroplasia) while her parents presented with the typical (mild) clinical signs of this disorder. Molecular analysis of DNA from the patient revealed compound heterozygosity for the N540K and the N540S exchange. As expected, the parents are heterozygous for one of the mutations each. To our knowledge this is the first report of a compound heterozygosity for HCH mutations. Clinical and molecular findings of the three affected family members are discussed.

**A Decade of Marfan Syndrome.** *D.M. Gilchrist.* Dept Medicine, Univ Alberta, Edmonton, AB., Canada.

**Objective:** To review a decade of experience in Marfan Syndrome in the Northern Alberta Medical Genetics Clinic (catchment approx. 1 million) **Method:** All patients were reviewed by a single geneticist who used diagnostic criteria outlined in the Berlin Nosology (1988) and, subsequently, the Revised Criteria (1996). **Results:** Of all referrals for inherited disorders of connective tissue, 49 patients from 30 families were felt to have definite Marfan and 43 patients from 28 families were felt to have possible Marfan. Of the definite cases, the average age at diagnosis was 27 with 4 being <10 (all from known families) and 13 of ages between 10 and 18 (5 from known families). Family history was secure for 32 patients in 15 families (50%). In those with family history, only 4 patients had major criteria in eye, heart and skeleton. In all patients with family history, 8 had ectopia lentis whereas 26 had aortic dilatation and/or dissection. Of the cases without family history, 6 had major findings in 3 systems and 1 had minor eye with major heart and major skeleton. Ten adults without family history had major findings in heart and skeleton only. Although these latter patients do not formally meet criteria, we consider them to have Marfan Syndrome. On analysis by feature of all definite cases, 25% had ectopia lentis and 84% had aortic dilatation/dissection. Most reliable MSK features included joint hypermobility (79%), dolicoostenomelia (67%), some degree of pectus deformity (68%), pes planus (58%), and striae (50%). Of 43 patients from 28 families with possible Marfan, major skeletal features with family history of same occurred in 18 from 8 families and mild aortic changes plus skeletal features occurred in 13 from another 8 families. **Interpretation:** Diagnostic criteria for Marfan syndrome are helpful but in the absence of family history and ocular findings, the diagnosis of Marfan Syndrome can still be compelling. We find major findings of the heart and multiple findings in the skeleton to be of more assistance than ocular findings in the diagnosis of patients presenting in adult years for Marfan Syndrome.

**Malignant peripheral nerve sheath tumors in neurofibromatosis 1.** *D.G.R. Evans<sup>1</sup>, M.E. Baser<sup>2</sup>, J.M. Friedman<sup>3</sup>, J. McGaughran<sup>1</sup>, B. Timms<sup>4</sup>, A. Moran<sup>4</sup>.* 1) Department of Medical Genetics, St. Mary's Hospital, Manchester, U.K; 2) Los Angeles, CA, U.S.A; 3) Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada; 4) Centre for Cancer Epidemiology, Manchester University, Manchester, U.K.

Most cross-sectional studies have shown that 1-2% of people with neurofibromatosis 1 (NF1) develop malignant peripheral nerve sheath tumors (MPNST). However, no population-based longitudinal studies have assessed lifetime risk. We ascertained MPNST patients from the North West (U.K.) regional NF1 genetic register and from reviewing notes of patients with MPNST in the North West Regional Cancer Register (regional population, 4.1 million) for the period 1984-1996. Twenty-four NF1 patients developed MPNST (equivalent to an annual incidence of 1.6 per 1,000 and a lifetime risk of 8-13%), and there were 38 patients with sporadic MPNST. The median age at diagnosis in NF1 patients with MPNST was 26.3 years (range, 15.5-77.2 years), compared to 62.1 years (range, 18.8-89.4 years) in those with sporadic MPNST (Mann-Whitney U test,  $P < .001$ ). In Kaplan-Meier analyses, the median survival from diagnosis in NF1 patients with MPNST was 1.0 year (95% C.I., 0.0-2.0 years), compared to 2.7 years (95% C.I., 1.2-4.1 years) in people with sporadic MPNST ( $P = .09$ ). The five-year survival rate was 25% in NF1 patients with MPNST and 45% in patients with sporadic MPNST. One NF1 patient developed two separate MPNST in the radiation field for a previous optic glioma. These results indicate that the lifetime risk of MPNST in NF1 is much higher than previously estimated, and warrants careful surveillance for improved detection.

**Walker-Warburg Syndrome; a report of long term survival.** *N.J. Mendelsohn<sup>1,2,3</sup>, J. Roggenbuck<sup>1,2</sup>, C. Ludowese<sup>1,2</sup>, W.B Dobyys<sup>4</sup>, R. Kriel<sup>1,2,3</sup>, S. Smith<sup>2</sup>.* 1) Dept Pediatrics/Med Genetics, Hennepin Co Med Ctr/Univ MN, Minneapolis, MN; 2) Gillette Children's Hospital, St. Paul, MN; 3) Dept. of Pediatrics, University of Minnesota; 4) University of Chicago, Chicago Illinois.

Walker-Warburg syndrome (WWS) is an autosomal recessive disorder marked by severe cobblestone complex brain malformation, anterior chamber and retinal malformations, and congenital muscular dystrophy. The brain abnormalities consist of severe cobblestone cortex (lissencephaly), abnormal white matter, enlarged lateral ventricles often with hydrocephalus, severe brainstem and cerebellar hypoplasia, and sometimes occipital cephaloceles. WWS is similar to but more severe than muscle-eye-brain disease (MEB), but maps to a different locus. Infants with WWS are considered to have extremely poor prognosis with severe mental retardation and limited survival. Dobyys et al. (1989) established diagnostic criteria, which have recently been revised (Cormand et al., 2001). A median survival for all liveborn infants has been reported to be 18 weeks, although two patients reported by Cormand et al. (2001) survived to age 3 years. A few of the affected children were noted to survive more than 5 years, all described as individuals with less severe mental retardation, reaching the milestones of rolling and sitting between 1 and 3 years of age. Recent review of these cases indicates that all probably had MEB, a separate disorder. Kanoff (1998) et al reported 2 children with WWS. The first died at 6 months of age and the second child survived beyond 18 months. He attributed the shorter survival time of the first patient to more severe myopathic changes. We report a young girl now 5 years of age (date of birth 9/9/1996), who was born with aqueductal stenosis, hydrocephalus, cobblestone lissencephaly, optic nerve colobomas, and congenital muscular dystrophy. MRI changes were typical of WWS and not MEB. She has profound developmental delay, yet clear evidence of survival well beyond the first year of life. This case suggests that survival in WWS may extend into the school age years and may correlate with factors other than severity of mental retardation, such as severity of hydrocephalus, seizures and myopathy.

**Natural history of Gelophysic Dysplasia: report of one case with 30 years old.** *S.F. Mesquita<sup>1</sup>, D.R. Bertola<sup>2</sup>, L.M.J. Albano<sup>2</sup>, A.C. Pereira<sup>1</sup>, J.E. Krieger<sup>1</sup>, C.A. Kim<sup>2</sup>.* 1) INCOR, Instituto do Coracao, Lab Genetica e Cardiologia Molecular, Sao Paulo, Brazil; 2) Genetics, Instituto da Crianca, University of Sao Paulo, Brazil.

Spranger et al. (1971), characterized geleophysic dwarfism: pleasant and happy-looking faces, small stature and dysostosis-multiple-like changes, predominantly in the hands and feet. A metabolic pathogenesis was suspected, but has not yet been identified. Koiffmann et al (1984) reported the disorder in a 11-year-old Brazilian girl. She was born at home, short with small hands and feet. She was the product of the 2nd pregnancy of her mother, healthy, young and nonconsanguineous parents. The first child was small with tiny hand and died at 3 years of cardiac failure. The patient had 2 normal sisters and 2 normal brothers. Clinical findings at 11 years old were a peculiar but pleasant and happy-looking facial appearance, with "tip-toe" gait because of bilateral talipes equinovarus, joint limitation at the elbows and hepatomegaly. She had an important subaortic stenosis and left ventricle hypertrophy. We report on the same patient at 30 years old with favorable evolution. She is still short stature (1,42 m), 42 kg, microcephaly (OFC=51 cm), same pleasant and happy facies, thin upper lip, small ears, high-pitched voice, short arms with elbow restriction, brachydactyly, small hands and feet, "tip-toe" gait, atrophic left limb. She had normal sexual and mental development. She was submitted to a subaortic fibrous ring resection and partial left ventricle muscle resection, at 24 years old. This proceeding improved her cardiac performance with normal ventricle function. Current echocardiography shows mild left ventricle hypertrophy and mild aortic insufficiency. Both left and right ventricle function are normal. Interestingly, although geleophysic dysplasia is suggested to be a lysosomal storage disease, no clinical progression was evident. Therefore, more aggressive and surgical corrections should be indicated to improve prognosis and quality of life of these patients.

**The clinical significance of heteromorphism chromosome 4p10.** *T.I. Datkhaeva, E. Sujansky, C.-H. Tsai.* Genetics and Metabolism, University of Colorado School of Medicine; 1056 East 19th Avenue, Denver, CO 80218.

Heteromorphic sites have been reported on many chromosomes, including chromosome 4p. We report a mother and 3 children with developmental delay (DD) and heteromorphic sites on chromosome 4p10. Heteromorphic sites have been reported in normal population (8%). However, they were found with higher frequency in people with congenital malformations (Lubs et al., 1976), in parents of children with chromosome abnormalities (Nielsen et al., 1974). Bardhan et al. (1981) compared 8% rate of heteromorphism of chromosome 4 in general population with 36% rate of heteromorphism in mental retardation, 43% in developmental delay and hyperactivity and 40.9% in schizophrenia. Barr et al. (2000) reported higher polymorphism in ADHD and mental illness, Vincent (1998) in obesity and NIDDM, Inoe et al. (1997) in cerebellar ataxia and MR, Faivre et al. (2000), Cotton et al. (1998) and Kennedy et al. (1998) in alcoholism. Although chromosomal heteromorphism has been studied extensively, its clinical significance is unclear. In our family all 4 members had heteromorphism of 4p10. The mother's IQ was reportedly <70. Her 1st child died of SIDS. His karyotype was unavailable. Three other children are alive. Her two sons age 4 years and 1 year respectively are developmentally delayed and dysmorphic. Her daughter was morphologically and developmentally normal at age 1 month except nipples asymmetry, but reportedly hypotonic at age 2 months. Unaffected family members were not available for chromosomal analysis. Our findings support the hypothesis that a causal relationship may exist between presence of the heteromorphic sites and developmental problems and/or malformations. It has been suggested that heteromorphic regions contain polygenes. The high frequency of heteromorphia in birth defects, developmental, mental and physical abnormalities suggest that the heteromorphia may be one of the factors contributing in polygenic disorders.

**GASTROSCHISIS AND OMPHALOCELE IN THE GENETICS CLINIC.** *B.G. Kousseff.* Pediatrics and Genetics, USF Regional Genetics Program, Tampa, FL.

Since 1960s improved definition, diagnosis, and reporting of gastroschisis (G) and omphalocele (O) have shown that the frequency of gastroschisis is on the increase. While most large studies document this increase, the textbooks in English still state that O (incidence of 1:4,000) is more common than G (incidence 1:6,000). Retrospective analysis of all 36,665 probands/families evaluated at the USF genetics clinic between January 2, 1982 and December 31, 1999 showed 121 patients with G and 98 with O. The higher number of G reversed the customary G: O incidence ratio of 1/6,000:1/4,000. Of the G patients 37/121 had karyotypes, all were normal. In the O cohort 75/98 had karyotypes and 18 were abnormal (24%); 12/18 had trisomy 18, 2/18-trisomy 21, 1/18-trisomy 13, 1/18-del (18p)/i(18q), 1/18-inv (2)(p11q12)mat and 1/18-inv (3)(p13q11) mat. In the G cohort 43/121 (36%) had associated anomalies compared to the O cohort with 67/98 (68%). 5/121 pregnancies with G were interrupted; 2/121-miscarried and there were 2 stillbirths. 33 pregnancies remained with unknown to us outcome (lost to reevaluations. Among the 79 liveborn the prematurity rate was 57%. 11/98 O pregnancies were electively interrupted; additional 6/98 miscarried and 23 remained with unknown outcome. Among the 58 liveborn patients the prematurity rate was 36%. The mortality rate in the G cohort was 9% (7/79) and in the O cohort it was 15% (9/58).

This study contributed to several issues in these abdominal defects: G is more common than O, has lower mortality rate, and higher rate of prematurity compared to O.

**International network to investigate the genetics of Chronic Obstructive Pulmonary Disease.** *A. Ashley-Koch, A. Agusti, P. Calverley, H. Coxon, C. Donner, B. Make, N. Muller, P. Pare, S. Rennard, J. Vestbo, E. Wouters, A. Franklin, M. Hall, C. Haynes, J. Butrim, M.A. Pericak-Vance, L.T. Middleton, C. Foster, E.K. Silverman, D.A. Lomas.* All on behalf of the Genetics of Chronic Obstructive Pulmonary Disease International Network.

Chronic Obstructive Pulmonary Disease (COPD), including emphysema and chronic bronchitis, is a major public health concern, affecting over 44 million people worldwide. The etiology of COPD is complex, with both genetic and environmental factors implicated. Alpha1-antitrypsin deficiency is the only well-established genetic factor, but accounts for only 1-2% of all emphysema cases. Cigarette smoking is the most significant environmental risk factor, although smoking history explains only 15% of the variability in forced expiratory volume (FEV1), and only 10-20% of smokers develop COPD. The goal of the present study is to identify additional genetic risk factors that contribute to COPD susceptibility. We have ascertained thus far 423 COPD families, without A1AT deficiency, across 10 international sites. These families include 169 independent affected sibpairs with airflow obstruction (FEV1<80%, FEV1/VC<90% predicted). Of the probands for whom ascertainment has been completed (n=335), 59.4% are male, median age is 59.0 (range 45-65), median FEV1% predicted is 31.3 (range 13.3-59.7), median FEV1/VC% predicted is 42.8 (range 19.0-89.0), and median pack-years smoking is 46 (range 5.75-159.5). Among smoking siblings, 36% met the criteria for airflow obstruction. Of these completely ascertained affected siblings (n=194), 55.2% are male, median age is 57 (range 44-66), median FEV1% predicted is 53 (range 24.0-79.6), median FEV1/VC% predicted is 68.0 (range 36.7-89.0), and the median pack-years smoking is 41.0 (range 6.0-156.0). In addition to standard clinical and environmental variables, both objective and subjective analysis of High Resolution CT scans are performed on all siblings > 40 years of age with at least a 5 pack-year smoking history. Preliminary HRCT analysis suggests that as many as 29% of asymptomatic siblings can exhibit significant emphysema. These data will be used to stratify sub-phenotypes in a genomic screen.



**Familial pleural fibrosis with ocular telangiectasia and male infertility: a new syndrome?** *E. Bieth<sup>1</sup>, N.*

*Chassaing<sup>1</sup>, R. Mieusset<sup>2</sup>, M. Benahmed<sup>3</sup>, J. Bienvenu<sup>4</sup>, H. Hamel<sup>5</sup>, P. Calvas<sup>1</sup>, D. Lauque<sup>6</sup>.* 1) Genetique, hopital Purpan, Toulouse, France; 2) CECOS, hopital La Grave, Toulouse, France; 3) Inserm U407, faculte de medecine Lyon-sud, Lyon, France; 4) Immunologie, hopital Lyon-sud, Pierre-Benite, France; 5) Centre hospitalo-universitaire, Dijon, France; 6) Medecine interne, hopital Purpan, Toulouse, France.

We report on three sibs, two sisters and a brother affected with a progressive bilateral pleural fibrosis. The parents were first cousins and the siblings had identical symptoms with similar severe evolution suggesting autosomal recessive inheritance. The brother was the oldest (30 years) and the most severely affected with a severe restrictive pattern of the pulmonary function testing. Chest computed tomography showed bilateral apical pleural thickening. As his two sisters he had conjunctival telangiectasia. In addition, he had an infertility due to a secretory oligoasthenospermia. None of the sibs were exposed to mineral dusts.

Because of his demonstrated involvement in fibrotic diseases of the lung as asbestos and in angiogenesis, the transforming growth factor beta (TGF- $\beta$ ) was investigated. It has been also shown that TGF- $\beta$  is expressed in human seminiferous tubules. The TGF- $\beta$ 1 levels in serum, supernatants of cultured skin fibroblasts and seminal fluid were normal. Furthermore, genetic analysis of three polymorphic markers linked to the TGF- $\beta$ 1 failed to find homozygosity in the three sibs suggesting that the TGF- $\beta$ 1 gene was not directly implicated. On the other hand, we hypothesized that the disease of the siblings could be due to mutations affecting one component of the TGF- $\beta$ 1 pathway. However, genotypic analysis of markers linked to several Smad genes was not conclusive to date. Other candidate gene studies coupled to homozygosity mapping are in progress.

**DYSMORPHOLOGICAL ANALYSIS OF FETAL ALCOHOL SYNDROME (FAS) CLINIC REFERRALS: A REPORT OF 300 CASES RECEIVING MULTIDISCIPLINARY EVALUATION.** *R.D. Blackston<sup>1</sup>, C.D. Coles<sup>2,3</sup>, J.A. Kable<sup>3</sup>, K.K. Howell<sup>2</sup>, J. Bertrand<sup>2</sup>, B. Meeks<sup>3</sup>, J. Haar<sup>3</sup>.* 1) Div Med Gen, Dept Pediatrics, Emory Univ Sch Medicine, Atlanta, GA; 2) Department of Psychiatry at EUSM, Atlanta, GA; 3) Marcus Institute a Division of the Kennedy-Krieger Institute at EUSM, Atlanta, GA.

We report initial dysmorphological and demographic data from a cohort of 300 referrals to a large FAS Clinic. The cohort was 51%- Caucasian, 46%-African-American (A-A) and 3%- other. The male/female distribution was 55.4% to 44.6%. Average age at evaluation was 5.3 years (SD: 3.4 yrs) with a range of 0 to 20 years. Most (83%) were not living with biological parents with 50.1% in foster care. Neonatal problems were noted frequently (57.6%) and birthweight was in the SGA range (M=2412 GMS). Growth (height, weight, and head circumference) appeared to normalize over time. Subjects were examined using a 30-item Dysmorphia Checklist. Findings of greater than 10, plus positive maternal history of alcohol use yielded, a partial FAS diagnosis. Scores >15 resulted in a FAS diagnosis. Thirty-two referrals were found to have no evidence of prenatal alcohol exposure or symptomatology consistent with FAS. While dysmorphia scores dropped over age, certain features persisted, particularly, hypoplastic philtrum and increased inner-ocular distance. Using a univariate ANOVA, there was a significant effect of ethnicity with AA's, (15.00 vs 11.16) having higher mean dysmorphic scores. Observed amelioration of alcohol effects on physical characteristics may be accounted for in several ways. Alteration in dysmorphia may occur with age and growth. Improved height, weight, head size are likely attributed to improved living environments outside the biologic home, associated with better nutrition, medical care, early intervention services, and consistent parenting, as opposed to neglect, abuse, undernutrition, poor medical care, addiction, and violence characteristics of many alcoholic homes. Further studies are underway to validate initial positive results.

**The long-term effects of prenatal exposure to anti-epileptic drugs.** *U. KINI, J. Clayton-Smith and The Liverpool and Manchester Neurodevelopmental Study Group.* Dept. of Clinical Genetics, St. Mary's Hospital, Hathersage Road Manchester, Lancashire, United Kingdom.

**Objective:** Children born to mothers with epilepsy are at a 2-3 fold increased risk of congenital abnormalities. This has been attributed in part to exposure to specific antiepileptic drugs (AED) in utero. However the effects of these drugs on neuropsychological development is less clear. The purpose of our study is to examine the long-term effects of prenatal exposure to AED with regards to dysmorphic features, structural abnormalities, cognitive impairment and behavioural problems. **Methods:** In a retrospective study of 218 mothers who had attended regional epilepsy clinics in the last 10 years, we assessed 370 children exposed to different AEDs. Structured interviews, hospital records, clinical examination and psychometric tests such as NART (maternal IQ), WISC (child's IQ) and the Rivermead memory test (memory) were the tools used in the study. **Results:** Of the 370 children examined: 62 were exposed to valproate (VPA) monotherapy, 96 to carbamazepine (CBZ) monotherapy, 73 to polytherapy and 100 were non-exposed (NE). Those exposed to VPA had significantly more dysmorphic features (median 5) in contrast to the CBZ group (median 2,  $p=0.01$ ) and to the NE group (median 2,  $p=0.05$ ). Major malformations were also more common in the VPA group - 24.2% (Odds ratio 3.7, CI 1.4-9.2). Those exposed to VPA monotherapy had a significantly lower mean verbal IQ (82.26) than CBZ (94.63) and NE groups (91.81) at the  $p<0.05$  level. Also, VPA monotherapy resulted in the lowest full scale IQ when compared to all groups. Memory impairment and special educational needs (SEN) were more common in the VPA group, with only 41% having normal memory function and 42% registered as having SEN. Behavioural problems such as poor attention span and hyperactivity were more commonly reported in the VPA group. **Conclusion:** Prenatal exposure to VPA, although confounded by seizure type and frequency, appears to result in increased dysmorphism, structural abnormalities, cognitive impairment and a higher incidence of behavioural problems and SEN. This has significant implications for the management of women with epilepsy.

**Parkinsonism phenotype in a large Azorean family affect with Machado-Joseph Disease.** *L. Mota-Vieira<sup>1</sup>, A. Ferro<sup>2</sup>, I. Silveira<sup>2</sup>, J. Sequeiros<sup>2</sup>, P. Coutinho<sup>3</sup>, J. Lopes<sup>1</sup>.* 1) Hosp. Divino Espirito Santo, Ponta Delgada, Azores Islands, Portugal; 2) UnIGENE, IBMC - Univ. Porto, Porto, Portugal; 3) Hosp. São Sebastião, Feira, Portugal.

Machado-Joseph Disease (MJD/SCA3) is an autosomal dominant neurodegenerative disease characterized by different clinical forms (types I - IV). Here we present a clinical and genetic study of a large MJD family from the Azorean island of São Miguel (Portugal), with 8 affected individuals through five generations. In this family there are at least 2 patients with the usual clinical phenotype of late onset of MJD (type III), and 2 patients, in consecutive generations, with the rare early onset parkinsonism phenotype associated with MJD (type IV). The propositus (V generation) is a 31-year-old woman. At age 30 she showed parkinsonism features (mild bradykinesia with weakness of spontaneous facial movements, diminished blinking and slowness of all voluntary movements) associated with hypertonia/rigidity, impaired postural reflexes and peripheral neuropathy. Her mother (IV generation) also developed an early onset parkinsonism at age of 35. Both of them obtained substantial benefit from L-dopa (levodopa-carbidopa) therapy. The maternal grandfather (III generation) and great-grandmother (II generation) noted only an onset of difficulty walking at ages 65 and 60, respectively. In order to obtain a pathological confirmation of MJD in this family, we analyzed the (CAG)<sub>n</sub> tract localized on the *MJD1* gene of the propositus. This analysis revealed an expanded allele and a normal allele with 74 and 15 CAG repeats, respectively (mutated alleles, 56-86 repeats; normal alleles, 7-47 repeats). This finding confirms that parkinsonism phenotype associated with peripheral neuropathy could be caused by MJD/SCA3 mutation. Moreover, the identification of this mutation will facilitate the genetic counseling in this family, in which the inheritance of MJD shows an anticipation of 30 years from type III to type IV. However, the reason(s) for different MJD phenotypes in the same family is(are) not known. The possible explanations will be discussed. (lmv.hospdelgada@mail.telepac.pt).

**Ultrasound analysis of orbicularis oris muscle defects in individuals with cleft lip with or without cleft palate and their relatives.** *S.M. Weinberg<sup>1</sup>, K. Neiswanger<sup>1</sup>, M.P. Mooney<sup>1</sup>, R.S. Faix<sup>2</sup>, D.A. Richardson<sup>2</sup>, S.S. Petiprin<sup>1</sup>, K.M. Bardi<sup>1</sup>, R.F. Giles<sup>1</sup>, E.S. Carter<sup>1</sup>, A. Bowen<sup>2</sup>, M.L. Marazita<sup>1</sup>.* 1) School of Dental Medicine, University of Pittsburgh, Pittsburgh PA; 2) Childrens Hospital, Pittsburgh PA.

Every year, oral-facial clefts affect between 1 in 500 to 1000 births worldwide. Despite this fact, only a few genetically linked phenotypic markers have been identified. Recently, however, a sub-clinical defect of the orbicularis oris (OO) muscle, was detected through ultrasound in the first-degree relatives of children with CL/P. Furthermore, these first-degree relatives displayed significantly more OO defects compared to controls ( $p=.002$ ). It was, therefore, hypothesized that these sub-epithelial clefts may represent a useful feature for estimating recurrence risks. Unfortunately, prior ultrasound analyses of OO have been restricted to first-degree relatives in non-multiplex families and failed to characterize the defect. Therefore, as part of a large multiplex family study on the cleft phenotype and genotype, ultrasound images were collected from affected (CL/P) probands and their affected and unaffected extended relatives ( $n=66$ ). The goal of this preliminary study was to develop a protocol for the analysis of OO ultrasounds and to compare data collected from unaffected relatives ( $n=43$ ) to unaffected controls ( $n=52$ ). Due to a lack of normative control data at this time, however, figures from the literature were utilized for comparison. The images were rated for defects on a scale from 1 to 4 (1 = normal, 2 = thinning, 3 = minor defect, and 4 = major/multiple defects) independently by three raters. The combined proportion of relatives with OO defects (rated as 2, 3 or 4 by at least two raters) was then calculated. The results of chi-square analysis revealed significant ( $\chi^2=12.9356$ ;  $DF=1$ ;  $p<.001$ ) differences in the proportion of unaffected relatives (44%) with OO defects compared to controls (11%). These results strongly agree with previous figures from the literature and lend support to the notion that such sub-clinical defects may indicate an increased susceptibility to CL/P. NIH grants DE13076 and RR00084.

**Maturity onset diabetes mellitus in a patient with maternal uniparental disomy for chromosome 14. T.**

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We report a 20-year-old man with maternal uniparental disomy for chromosome 14 [upd(14)mat] and diabetes mellitus, the second case reported with such an association. The first such instance was a 19-year-old man described by Carozzo (Clin Genet 57;406-408, 2000) with probable maternal isodisomy for the entire length of chromosome 14, but the diagnosis was uncertain in the absence of genetic studies of the father. The man we are reporting had pre- and postnatal growth retardation, and at age 20 years developed diabetes mellitus without any autoimmune antibody. His chromosomes were 45,XY,der(14;14)(q10;q10)[29]/46,XY,+14,der(14;14)(q10;q10)[1]. Studies of CA-repeat markers covering the entire length of chromosome 14 indicated maternal isodisomy of the 14q21-q24 segment and maternal heterodisomy of the remaining regions. It is thus tempting to speculate that homozygosity of a mutant gene at the isodisomic segment caused his diabetes mellitus. The IDDM11 gene is such a candidate with its locus situated close to the segment.

**Post-zygotic somatic recombination between maternal and paternal #13 in a child with Angelman syndrome and facial findings of Trisomy 13 homologues.** *C.H. Tsai<sup>1</sup>, T. Gibby<sup>1</sup>, L. Beischel<sup>3</sup>, L. McGavran<sup>2</sup>, J.P. Johnson<sup>3</sup>*. 1) Dept Pediatrics/Div Genetics, Childrens Hosp, Denver, CO; 2) Colorado Genetics Laboratory, Departments of Pathology/Pediatrics, UCHSC; 3) The Molecular Genetics Laboratory at Shodair Childrens Hospital, Helena, Montana.

We describe a child with findings of Angelman syndrome and trisomy 13 with a paternally derived Robertsonian translocation of 13 and 15. Molecular studies showed paternal uniparental heterodisomy (UPD) 15 and segmental paternal isodisomic UPD 13. This 6- year old boy was seen for global developmental delay associated with a karyotype of 45,XY,t(13;15)(q10;q10). He was AGA born to a 25-year old G3P2 mother at 32 weeks by vaginal delivery. Bilateral grooved lip and cleft palate were noted at birth, with early feeding difficulties. There were no clinically recognized seizures. Brain MRI was normal. He is nonverbal. Physical examination showed a happy child with global developmental delay, normal growth, a deviated uvula, a scalp scar from cutis aplasia, bilateral Sydney lines, sandal gap, and a hypopigmented spot. Neurologically, he had hypertonia, clonic movements, and ataxic gait. FISH on 200 cells showed no evidence for mosaic trisomy 13 or 15. Paternal chromosome studies showed a balanced carrier, 45,XY,t(13;15)(q10;q10). UPD studies for chromosome 15 showed paternal uniparental heterodisomy for four informative loci consistent with the diagnosis of Angelman syndrome. UPD studies for chromosome 13 showed segmental paternal uniparental isodisomy for three informative loci at 13q14.3. Paternal UPD 15 and segmental UPD 13 consistent with a post-zygotic somatic recombination between maternal and paternal #13 homologues in Angelman syndrome has not been reported. This patients clinical findings point to a possible critical region for cleft lip and palate and cutis aplasia in 13q14.3 resulting from paternal segmental UPD13. These unusual results demonstrate the importance of recognizing characteristic physical features in making a syndromic diagnosis and of fully exploring such cases cytogenetically and molecularly to determine their genetic etiology.

**Maternal uniparental disomy 14 presenting as language delay.** *K.A. Worley<sup>1</sup>, V.R. Rundus<sup>1</sup>, E.B. Lee<sup>1</sup>, V.L. Hannig<sup>1</sup>, L.K. Hedges<sup>1</sup>, K. Tsuchiya<sup>2</sup>, J.A. Phillips III<sup>1</sup>.* 1) Dept Pediatrics, Vanderbilt Univ Sch Medicine, Nashville, TN; 2) Dept Pathology, Vanderbilt Univ Sch Medicine, Nashville, TN.

Maternal uniparental disomy of chromosome 14 (MUPD14) is a rare disorder characterized by low birth weight, hypotonia and feeding difficulties in infancy followed by short stature, small hands and early puberty. Affected individuals may have translocations involving 14. We report here a case of MUPD14 that presented at 3 years of age with severe language delay, normal intelligence, and short stature. Birth weight was < 3<sup>rd</sup> % and birth length was between the 10 - 25<sup>th</sup> %. He had neonatal hypotonia and was found to have a 45, XY, der (13;14) (q10; q10) translocation. He required gavage feeding and physical therapy. Gross motor skills were age appropriate at 12 months. His language was at the 20-24 month level at 36 months and speech therapy began. Height and weight at 41 months were < 3<sup>rd</sup> % (50<sup>th</sup> % for 2.3 and 1.45 years respectively) but his bone age was 4.5 years. Head circumference was normal and the only dysmorphic feature noted was short fingers (< 3<sup>rd</sup> % for newborns). Because of language delay, short stature, short fingers and the previous finding of a translocation (13;14), DNA studies were done for MUPD14. Results showed isodisomy of D14S261, 283, 275, 70, 288 and 292, heterodisomy of D14S74 and 280, and D14S 276, 63, 65 and 985 were partially informative. Chromosome 13 was biparentally inherited. The Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) (mean = 100; S.D. = 15) indicated verbal and nonverbal intellectual skills at 43 months were in the mid-average range (91 and 97 respectively with a Full Scale IQ of 93). The Vineland Adaptive Behavior Scales (mean = 100; S.D. = 15) suggested communication, daily living, socialization, and motor skills were in the low average to mildly delayed range (84, 83, 76, and 69 respectively). Our findings indicate that: 1) MUPD14 may present with language delay and subtle physical findings, and 2) careful evaluation of physical, chromosomal and developmental phenotypes may identify UPD in children presenting with the common complaint of language delay.



**A further delineation of the paternal uniparental disomy (UPD) 14: The fifth reported liveborn case.** *S. Yano<sup>1</sup>, L. Li<sup>1</sup>, S. Owen<sup>1</sup>, S. Wu<sup>1</sup>, T. Tran<sup>2</sup>.* 1) Div Medical Genetics, Childrens Hosp, Los Angeles and University of Southern California, Los Angeles, CA; 2) University Childrens Genetics Laboratory, ProGene, Inc.

Uniparental disomy (UPD) for chromosome 14 has been reported in more than 14 patients with maternal UPD and in 4 patients with paternal UPD (one additional case diagnosed prenatally and terminated, Cotter et al., 1999). Major clinical feature of maternal UPD 14 consists of premature puberty, developmental delay, hypotonia, short stature, small hands, IUGR, and macrocephaly/ hydrocephalus. At least three out of four patients with paternal UPD 14 had polyhydramnios, blepharophimosis, small ears, small thorax, abnormal ribs, feeding difficulty, digit contractures, and simian creases. We report the fifth liveborn case of paternal UPD 14. The proposita was born to a 16-year old G2P2 Hispanic female and a healthy unrelated Hispanic male. Polyhydramnios was noted prenatally. The proposita was delivered by Caesarian section at 32 weeks. She weighed 2200 grams. Multiple congenital anomalies were noted, which included loose skin, blepharophimosis, low set small ears, micro/ retrognathia, prominent glabella, short webbed neck, narrow small rib cage, abdominal wall hernia, and multiple joint contractures/camptodactyly. She developed a seizure disorder and was treated with phenobarbital. A brain MRI showed generalized hypoplasia and multiple small scattered areas of signal abnormality within the subependymal areas which may represent calcification. Parental chromosome studies showed normal karyotype in her mother and a balanced Robertsonian translocation 45,XY, der(13;14)(q10;q10) in her father. Linkage analysis using microsatellite markers on chromosome 14 (D14S742, D14S587, D14S606, D14S617, D14S129, and D14S267) revealed paternal UPD: analysis with D14S587 and D14S129 revealed uniparental isodisomy. This case provides further evidence of existence of imprinted genes on paternal chromosome 14 by showing common characteristic clinical features in the patients with paternal UPD 14.

**Autosomal dominant isolated cryptophthalmos (Parks-Saal)syndrome - a third family.** *V.M. Siu<sup>1,2,4</sup>, R.B. Orton<sup>3,4</sup>.*  
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Cryptophthalmos is a rare congenital anomaly of the eye, involving various degrees of fusion of the eyelids to the underlying cornea. The most well-known syndrome associated with cryptophthalmos is Fraser syndrome, an autosomal recessive syndrome, associated with mental retardation, syndactyly, and genital anomalies. Only 2 families with a dominant syndrome of cryptophthalmos have been previously reported in 1910 (Parks) and 1992 (Saal).

We present a female infant with bilateral incomplete cryptophthalmos born at term to nonconsanguineous parents - a 38 year old mother and 41 year old father. Eyebrows were well-defined and eyelashes were present around the lateral margin of the eyelids. Upper eyelids were bulging, thickened, and adherent to the underlying corneas, with a distinctive dimpling and horizontal groove in the eyelids, particularly prominent when the baby cried. Lower eyelids were partially separated, with the inferior fornices present. Superior lacrimal puncta were displaced laterally within the upper eyelids. MRI findings revealed a right microphthalmos with probable retinal detachment and normal-sized left eye with optic pit. A perianal skin tag and syringomyelia constitute the only other non-ophthalmological findings. At four months of age, the baby had normal growth and development, and light perception. Chromosome karyotyping was normal, including multitelomere FISH studies. Father has mild strabismus but normal vision.

Our patient's eye anomalies are similar to the 5 cases in 2 previously reported families. There have been no cases of male-to-male transmission, therefore the mode of inheritance may be either autosomal or x-linked dominant. Dimpling of the eyelids, normal eyebrow development, and absence of other major congenital anomalies allow rapid differentiation from Fraser syndrome. Developmental arrest of normal eye development is postulated.

**FAMILIAL IRIDOGONIODYSGENESIS AND SKELETAL ANOMALIES A NEW AUTOSOMAL RECESSIVE DISORDER.** *L.X. Rodriguez-Rojas<sup>1,4</sup>, R. Mendoza-Topete<sup>2</sup>, M.T. Barrios<sup>3</sup>, B. Patiño-Garcia<sup>1,4</sup>, M.G. Lopez-Cardona<sup>1,4</sup>, I. Nuño-Arana<sup>1,4</sup>, J.E. Garcia-Ortiz<sup>1,4</sup>, D. Garcia-Cruz<sup>1,4</sup>.* 1) Division de Genetica, CIBO-IMSS, Guadalajara, Mexico; 2) Hospital de Especialidades, CMNO-IMSS, Guadalajara, Mexico; 3) Hospital de Pediatria, CMNO-IMSS, Guadalajara, Mexico; 4) Doctorado en Genetica Humana, CUCS-UdeG, Guadalajara, Mexico.

Three sibs with congenital glaucoma and skeletal anomalies were studied. At birth enlarged eyes and cornea were present in the proposita and her brother. The younger sib presented blue cornea since birth. The proposita, aged 30 years old and a brother aged 27 years were submitted to several trabeculotomies, the other one aged 22 years developed unilateral buphthalmos. Clinically, they showed wide forehead, thin nasal bridge, broad nose with large tip, prominent eyes, long philtrum, large ears, wide thorax and cubitus valgus. The ophthalmological examination in the proposita revealed glaucoma, megalocornea, wide anterior chambers and concave iris. At gonioscopy, angle grade IV, with abundant fine iridian processes reaching Schwalbes line, minimum pigment and hypoplasia of the iris stroma. There was no evidence of posterior embryotoxon. One sib presented unilateral ptisis bulbi, cornea with grooves in Descemet, iris hypoplasia and corectopia (presenting blindness since 8 years old). The younger brother is blind since birth, he showed unilateral ptisis bulbi, without ocular tissues, contralateral buphthalmos, edematous cornea with central leukoma, whittish keratopathy in lower band. The propositas daughter and her parents were ophthalmologically normal. The radiological examination showed slender long bones with wide metaphyses and thin cortices, cuboid-shaped vertebral bodies, narrow vertebral canal, small pelvis, and generalized osteopenia, more evident at the metaphyseal region. We concluded that these patients present the clinical, ophthalmological and radiological characteristics as the family previously described by Garcia-Cruz et al (*Ophthal Paediat Genet*, 11(1): 35-40, 1990). The purpose of this paper is to describe the second familial case corroborating this new probably autosomal recessive disorder.

**Two cases of Xq28 functional disomy detected using subtelomeric probes screening.** *M.C.de Blois, D. Sanlaville, M. Prieur, J.M. Lapierre, C. Ozilou, P. Gosset, A. Munnich, V. Cormier-Daire, S.P. Romana, M. Vekemans, C. Turleau.* Departement de Genetique, Hopital Necker-Enfants Malades, Paris, France.

We report on two patients with an additional Xq28 segment attached to the long arm of an autosome. The first patient, a boy, was part of a cohort of patients with unexplained mental retardation and a normal karyotype at the 550 bands level. CGH screening showed an enhancement of band Xq28. Further studies with WCP and subtelomeric probes demonstrated that the additional Xq28 segment was translocated onto 10qter. The translocation breakpoint was found distal to the 10q subtelomeric probe which was not deleted. This unbalanced translocation  $der(10)t(X;10)(q28;qter)$  arose de novo. The second patient is a girl for whom a telomeric rearrangement of 4q was suspected on high-resolution karyotypes. Only one hybridization signal was present with the 4q subtelomeric probe. Using a 41-subtelomeric probes set, an additional signal was observed at the 4q end with the Xq probe. This unbalanced translocation  $der(4)t(X;4)$  arose de novo. In both cases, the unbalanced translocation resulted in a functional disomy for Xq28 which may explain the clinical manifestations observed in both patients. Ten boys with duplicated distal Xq segment and functional disomy have been described in the literature, but, to our knowledge, this is the first report of a girl with an unbalanced translocation yielding functional disomy for Xq28. When the phenotypes of both patients are compared to those of the literature, a particular dysmorphism with wide face and microstomia associated to severe mental retardation can be described. It is suggested that this rare rearrangement is presumably underdiagnosed and that subtelomeric probe screening could help to its detection.

**A new X-linked syndrome with similarity to CHARGE association, Toriello-Carey syndrome, and FG syndrome.**

*J.M. Graham Jr<sup>1</sup>, P. Wheeler<sup>2</sup>, A.E. Lin<sup>3</sup>, B.D. Hall<sup>4</sup>, T.C. Cox<sup>5</sup>, C.E. Schwartz<sup>6</sup>.* 1) Medical Genetics Birth Defects, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) Tufts-New England Medical Center, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) University of Kentucky Medical Center, Lexington, KY; 5) Centre for Molecular Genetics of Development, University of Adelaide, Australia; 6) Greenwood Genetic Center, Greenwood, SC.

We describe 2 brothers with a unique pattern of malformations that includes coloboma (iris, optic nerve), high forehead, severe micrognathia, mental retardation, and agenesis of the corpus callosum (ACC) that was initially thought to be CHARGE association. Both boys have low-set cupped ears with sensorineural hearing loss, normal phallus, pectus excavatum, and short stature. One brother has scoliosis, and the other has choanal atresia and VSD/ASD. The possibility of X-linked CHARGE-like syndrome (MIM # 302905) was considered, but this condition with cleft palate, coloboma, coronal hypospadias, deafness, short stature, and radial-ulnar synostosis with normal IQ was excluded because the latter 2 features were not present. Their facial features resemble an autosomal recessive syndrome described by Temtamy et al. (1996), which consists of ACC, mental retardation, micrognathia, low-set ears, and coloboma; however, the absence of brachydactyly, bulbous thumbs, genu vara, and pes planus differentiates these brothers from Temtamys siblings. Because of the ACC and marked micrognathia, there is some similarity to Toriello-Carey syndrome. Because of ACC and distinctive facial features, FG syndrome was considered and DNA was analyzed for markers linked to the FG locus. Both brothers are concordant for markers spanning the presumed FG region in Xq13-q21, and both have an alteration in the 5'UTR of the Alpha 4 gene. This gene encodes a protein, which interacts with the Opitz syndrome protein, MID1. We are testing this alteration to see if it is a polymorphism and if it has any biological effect, as well as checking other similar patients for changes in this gene.

**A unique case of atypical Rett Syndrome: MECP2 mutation in a 47, XXX female.** *S.A. Hammer, N. Dorrani, N.C. Schanen.* Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA.

Rett Syndrome (RTT) is a neurodevelopmental disorder of X-linked dominant inheritance, characterized by severe mental retardation, seizures, hypotonia, stereotypic hand movements and absent language. RTT is caused by mutation in MECP2, a gene located on Xq28 and subject to X-inactivation. MECP2 encodes for methyl CpG-binding protein 2, a ubiquitously expressed protein that binds to methylated CpG dinucleotides and recruits transcriptional silencing complexes to promoter regions. Mutations in MECP2 are primarily de novo events in the male germ line and thus lead to an excess of affected females. Here we report the identification of a unique 47, XXX female who presented with features of atypical RTT and who carries a de novo MECP2 mutation, L100V. She exhibits a relatively mild RTT phenotype that is distinct from the 47, XXX phenotype; she had regression in skills at 20 months of age and currently at five years, she has no language and limited use of her hands, but is agile and exceptionally socially engaging. To investigate the source of her mutation, intronic polymorphisms in MECP2 were analyzed in the patient and her parents. Genotyping of several markers on the X chromosome indicate that the supernumerary X chromosome is paternally derived. X-inactivation patterns were determined by analysis of methylation of the androgen receptor locus, and indicated essentially random inactivation of maternally and paternally derived alleles. The co-occurrence of a sex chromosome aneuploidy and MECP2 mutation are likely chance occurrences and unrelated mechanistically. Supported by HD 37874 and T32HD7032.

**Genetic Heterogeneity of X-Linked Recessive Microphthalmia-Anophthalmia with Mental Retardation: Is Lenz syndrome a single disorder?** *D. Ng<sup>1</sup>, D. Hadley<sup>1</sup>, C. Tiff<sup>2, 3</sup>, L. Biesecker<sup>4</sup>*. 1) Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Childrens National Medical Center, Washington, DC; 3) Genetics of Development and Disease Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD; 4) Genetic Diseases Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD.

We identified an African-American family with six affected males exhibiting variable features of microphthalmia or anophthalmia, microcephaly, mental retardation, renal and urogenital anomalies. This phenotype was inherited in an X-linked recessive pattern. These anomalies are consistent with Lenz microphthalmia syndrome. Lenz microphthalmia syndrome (MAA (microphthalmia with associated anomalies) [MIM 309800]) was credited to Lenz for his description of a family in 1955. The features in that family included mental retardation, microphthalmia, high palate, anteverted ears, dental abnormalities, congenital cardiac defect, skeletal defects of the fingers and clavicles, unilateral renal aplasia, and cryptorchidism. Subsequent to the report by Lenz, fourteen additional case reports have been published. These reports show that anophthalmia and microphthalmia are part of a spectrum of ophthalmic malformations. Importantly, these reports also show that there is a wide range of associated extra-ocular malformations in X-linked syndromic microphthalmia (XLSM), which were assumed to be caused by mutational heterogeneity, but alternative theories have not been tested. Forrester et al.,[2001] have demonstrated suggestive evidence of linkage of MAA to Xq27 (LOD max 1.83). We have performed linkage analysis in this region and excluded it in the family reported here. We conclude that MAA is genetically heterogeneous with at least two loci on the X chromosome.

**Integrating the cytogenetic map with the draft human genome.** *W. Jang<sup>1</sup>, H.-C. Chen<sup>1</sup>, R. Yonescu<sup>2</sup>, V. Cheung<sup>4</sup>, N. Nowak<sup>5</sup>, B.J. Trask<sup>3</sup>, I. Kirsch<sup>2</sup>, T. Ried<sup>2</sup>, G.D. Schuler<sup>1</sup>.* 1) National Center for Biotechnology Information, NIH, Bethesda, MD; 2) National Cancer Institute, NIH, Bethesda, MD; 3) Fred Hutchinson Cancer Research Center, Seattle, WA; 4) Dep. of Pediatrics, Univ. of Pennsylvania, Philadelphia, PA; 5) Rosewell Park Cancer Institute, Buffalo, NY.

For many human genetic diseases, cancers, and developmental disorders, disease phenotypes have been associated with chromosomal regions, most often to a cytogenetic band(s). In these cases, to understand molecular defects associated with the disease phenotype, it becomes important to identify genes in a given cytogenetic band. To this end, we have started integrating the cytogenetic map with the sequence map.

For this cytogenetic to sequence map integration, we are making use of BAC or PAC clones that have been mapped cytogenetically by fluorescent in situ hybridization (FISH) and have sequence tags in the form of end-sequence, insert sequence or STSs. These clones were placed on the sequence map based on the alignment of the sequence tag on the genomic contig sequence, providing cytogenetic band information on a region of sequence contigs. In NCBI's genome build 22, 7266 FISH-mapped clones were placed on genomic sequence contigs, providing data points for integrating cytogenetic and sequence maps. This annotation can be accessed by (1) selecting "Clone" map on the MapViewer; or (2) through cytogenetic resource (<http://www.ncbi.nlm.nih.gov/genome/cyto>); or (3) through the CloneServer (<http://www.ncbi.nlm.nih.gov/genome/clone>).

The Clone map in the MapViewer displays the clone position on the sequence map, FISH data, and their sequence tags. The cytogenetic resource lists all the FISH-mapped clones and one can select clones based on FISH-mapped chr, FISH data source, clone distributor, or sequence tags. The CloneServer contains information about the status of sequencing of a clone, insert accessions, end-sequence accessions, FISH mapping, STS mapping, placement result on the contig, and clone distributors.



Program Nr: 747 from the 2001 ASHG Annual Meeting

**HC Forum®: an international telemedicine program in cytogenetics.** *O. Cohen, M.A. Mermet, J. Demongeot.*  
Genetic Lab/TIMC/UMR CNRS 5525, Grenoble Univ Medical Sch, La Tronche, France.

Familial structural rearrangements of chromosomes represent a risk of imbalance at birth, which could vary in a large range, making genetic counseling difficult. They also represent a powerful tool for increasing knowledge of the genome, particularly by studying breakpoints and viable imbalances of the genome. However their great diversity makes difficult phenotypes-genotypes correlations, since a very large sample is necessary. From a collaborative database, including data on more than 4100 families, we have developed a Web site called HC Forum (<http://HCForum.imag.fr>). It offers cytogeneticists assistance in diagnosis by showing in real time rearranged chromosomes according to the international nomenclature (ISCN 1995). For genetic counselors, it provides an assessment of the risk of imbalance at birth in reciprocal translocations or in pericentric inversions, by using statistical models. For researchers, interfaces exhibit the distribution of chromosomal breakpoints and of the genome regions observed at birth in trisomy or in monosomy. They allow requests making easier the finding of specific papers or biological material of interest. Since the most of data stay unpublished, we have recently developed an application allowing secured data submissions through Internet. The users can select a kind of the anomaly, the chromosomes involved and the breakpoint locations from graphical interfaces. Interactive pedigree drawing is available and karyotypes can be inputted. Individual data can be described from dysmorphological features according to the LDDDB thesaurus and from chromosomal and clinical pictures of the abnormalities. All these data are showed in a unique friendly interface and can be electronically submitted. After their validation they are definitively registered in the database, but each user can find all the files he previously submitted. After two years, more than 900 users from about 47 different countries already exist. HC Forum constitutes a bridge between diagnosis laboratories and genome research centers, and is a powerful toll for genetic counseling, clinical cytogenetics and for research workers. Contact: [Olivier.Cohen@imag.fr](mailto:Olivier.Cohen@imag.fr).

**Chromosomal instability syndrome of total PCS with mosaic variegated aneuploidy: Genetic homogeneity of the two Japanese infants.** *S. Matsuura*<sup>1</sup>, *E. Ito*<sup>1</sup>, *T. Ikeuchi*<sup>2</sup>, *T. Kajii*<sup>3</sup>, *K. Komatsu*<sup>1</sup>. 1) Dept. of Rad. Biol., RIRBM, Hiroshima Univ., Hiroshima, Japan; 2) Div. of Genet., MRI, Tokyo Med. Dent. Univ., Tokyo, Japan; 3) Hachioji, Tokyo, Japan.

We (Kajii, 1998) previously reported a chromosomal instability syndrome in two Japanese infants, characterized by premature separation of chromatids, in all chromosomes, called "total PCS" in >50% of their lymphocytes, and mosaic variegated aneuploidy. Clinical findings included severe pre- and postnatal growth retardation, profound developmental retardation, severe microcephaly, hypodysplasia of the brain, Dandy-Walker anomaly, uncontrollable clonic seizures, and development of Wilms tumor. Recently we analyzed cell cycle of primary skin fibroblasts from another two unrelated Japanese infants with the disease; MY1sk from a male infant and PCS1 from the male infant reported by Kawame et al. (1999), and found that the two infants' cells are insensitive to the colcemid-induced mitotic-spindle checkpoint (Matsuura, 2000). Here we report functional complementation studies of the two infants' cells. We established immortal cell lines; MY1skSVT and PCS1SVT, from the primary skin fibroblasts; MY1sk and PCS1 respectively by use of SV40 transformation and hTERT cDNA transfection. The infants' cell lines both showed abnormal response of mitotic indices after colcemid treatment, that was similar to that of parental primary cells, indicating no phenotypic alteration in mitotic-spindle checkpoint during an immortalization process. After somatic cell fusions, hybrid cells were analyzed for their response in mitotic indices after colcemid treatment. Immortal cell line GM7166VA7C was used as normal control cells. When infants' cells, either MY1skSVT or PCS1SVT, was fused with GM7166VA7C cells, all hybrid clones showed accumulation of mitotic cells in response to colcemid treatment. In contrast, the hybrid clones between MY1skSVT and PCS1SVT showed fewer mitotic cells and no clear peak in mitotic index. These results suggested genetic homogeneity in the two Japanese infants, and also suggested that the cellular phenotype of abnormal mitotic-spindle checkpoint might be autosomal recessive.

**Maria Cristina Terreros will make the presentation instead of G. Padula.**

Program Nr: 749

**PROTEIN CALORIC MALNUTRITION AND CHROMOSOMAL ALTERATIONS: A CASECONTROL STUDY.** *G. Padula*<sup>1, 2</sup>, *M.C. Terreros*<sup>3, 4</sup>, *M. Apestegua*<sup>1, 3</sup>, *S.A. Salceda*<sup>1, 2</sup>. 1) Universidad Nacional de La Plata, Argentina; 2) CONICET; 3) Comision de Investigaciones Cientificas (CIC), Argentina; 4) Department of Biological Sciences, Florida International University, Fl. USA.

The relation between protein-caloric malnutrition (PCM) and genetic damage has been studied in human beings and laboratory animals obtaining contradictory evidences. It has been found that children aged 1-60 months with severe PCM exhibited an increase of chromosomal aberrations (dicentric, gaps, isogaps, breaks) in peripheral lymphocytes and bone marrow cell cultures. These abnormalities persisted even after the children had attained normal height and weight. In order to evaluate the effect of the protein-caloric malnutrition on the chromosomal damage a total of 9 individuals (7 females and 2 males) aged 1-60 were studied. Cytogenetics preparations from peripheral blood cells were made according to routine protocols and fixed according to Islam and Levan (1987). Chromosome spreads were stained for C banding according to Summer (1972). Cytogenetics analysis were performed in coded slides by scoring the frequencies of dicentric chromosomes, gaps, isogaps, breaks and telomeric associations (TAS). Statistical analysis were performed by Epi Info 6.0. The results obtained to date show a statistical significance increase of chromosomal aberrations. This genetic damage as consequence of PCM can be explained by a failure of enzymatic mechanisms of DNA replication and repair.

**The involvement of Fanconi anaemia proteins in homologous genetic recombination.** *M.S. Meyn*<sup>1,2</sup>, *A. D'Andrea*<sup>3</sup>, *M. Grompe*<sup>4</sup>, *W. Wang*<sup>1</sup>. 1) Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Molecular and Medical Genetics, University of Toronto, Toronto, ON, Canada; 3) Pediatric Oncology, Dana Faber Cancer Institute, Boston, MA; 4) Molecular and Medical Genetics, OHSU, Portland, OR.

Fanconi anaemia is characterized by pancytopenia, malformations, cancer, genetic instability, and impaired fertility. Its etiology has remained elusive despite the isolation of multiple Fanconi anaemia (*FANCA*) genes. We now report that the *FANCD2* protein associates with recombination proteins on synaptonemal complexes in spermatocytes and that lack of a functional *FANCA* or *FANCC* gene disrupts meiotic chromosome pairing.

By studying surface spreads of mouse spermatocytes we found that *FANCD2* first forms discrete foci along the unsynapsed and synapsed axes of homologous chromosomes in zygotene spermatocytes. The number of *FANCD2* foci peaks in pachynema, the stage when reciprocal recombination occurs. Most *FANCD2* foci are coincident with foci of Rad51 and RPA, components of meiotic recombination nodules.

*FANCC*<sup>-/-</sup> and *FANCA*<sup>-/-</sup> mice are subfertile. We found that *FANCD2* staining is unusually diffuse in many *FANCC*<sup>-/-</sup> and *FANCA*<sup>-/-</sup> spermatocyte nuclei. Meiotic pairing in *FANCC*<sup>-/-</sup> and *FANCA*<sup>-/-</sup> spermatocytes is disturbed, as indicated by a 15-20 fold increase in the frequency of mispaired chromosomes. In addition, *FANCC*<sup>-/-</sup> spermatocytes have elevated frequencies of apoptosis.

Our results provide a direct demonstration of the *in vivo* association of a Fanconi anaemia protein with chromosomal DNA. The colocalization of *FANCD2* with recombination nodules in meiosis I suggests that *FANCD2* may be part of the machinery of homologous recombination. Our findings that both *FANCD2* foci formation and chromosomal synapsis are impaired in the spermatocytes of *FANCC*<sup>-/-</sup> and *FANCA*<sup>-/-</sup> mice suggest that *FANCD2* is required for optimal pairing of homologous chromosomes during meiosis and that *FANCC* and *FANCA* facilitate the functioning of *FANCD2*. Our data argue that dysfunctional recombination may be a fundamental defect in Fanconi anaemia.

**Identification and characterization of a *de novo* translocation t(8;14)(q22.3;q13) associated with Klippel-Trénaunay Syndrome (KTS), a vascular disease.** A.A. Timur<sup>1,2,3</sup>, P. Szafranski<sup>4</sup>, A. Sadgephour<sup>1,2,3</sup>, V. Jurecic<sup>5</sup>, J.K. Cowell<sup>1,6</sup>, A. Baldini<sup>5</sup>, D.J. Driscoll<sup>7</sup>, Q. Wang<sup>1,2,3</sup>. 1) Center for Molecular Genetics; 2) Department of Molecular Cardiology, Lerner Research Institute; 3) Center for Cardiovascular Genetics, Department of Cardiology, The Cleveland Clinic Foundation, Cleveland, OH; 4) Department of Pathology; 5) Department of Pediatrics (Cardiology), Baylor College of Medicine, Houston, TX; 6) Department of Cancer Genetics, Roswell Park Cancer Institute, Buffalo, NY; 7) Division of Pediatric Cardiology, Mayo Clinic, Rochester, MN.

Klippel-Trénaunay syndrome (KTS) is a vascular disease comprised of capillary, lymphatic, and venous malformations associated with limb asymmetry. We report the identification of a balanced translocation involving chromosomes 8q22.3 and 14q13 in a patient with KTS. We demonstrated that translocation t(8;14)(q22.3;q13) arose *de novo* and co-segregates with the disease in the KTS kindred. The identification of a *de novo* translocation associated with KTS strongly supports the hypothesis that genetic factors contribute to the development of KTS. Fluorescence in situ hybridization (FISH) analysis was used to define the breakpoint on chromosome 8q22.3 to a <5-cM interval flanked by markers AFMA082TG9 and GATA25E10, and the 14q13 breakpoint within a 1-cM region between STS WI-6583 and D14S989. Possible candidate genes for KTS were identified from the breakpoint regions through the NCBI GeneMap'98 database. The 8q22.3 breakpoint contains angiopoietin-1 gene encoding an angiogenic factor (*ANGPT1*) that is required for embryonic vascular stabilization, branching morphogenesis, and post-natal angiogenesis. FISH analysis indicated that the *ANGPT1* gene is not disrupted by the 8q22.3 translocation breakpoint. Further definition of the breakpoints may assist in the identification of a pathogenic gene causing KTS.

**Trisomy 15qter Including the IGF1 Receptor Gene and Overgrowth: Report of Two Families and Review of the Literature.** *P. Gosset<sup>1</sup>, L. Faivre<sup>1</sup>, V. Cormier-Daire<sup>1</sup>, I. Guirgea<sup>1</sup>, S. Odent<sup>2</sup>, J. Amiel<sup>1</sup>, MC. Nassogne<sup>3</sup>, A. Munnich<sup>1</sup>, S. Romana<sup>1</sup>, M. Prieur<sup>1</sup>, M. Vekemans<sup>1</sup>, C. Turleau<sup>1</sup>, MC. De Blois<sup>1</sup>.* 1) Dept of Genetics, Hopital Necker-Enfants Malades, Paris, France; 2) Dept of Genetics, Hopital Pontchaillou, Rennes, France; 3) Dept of Metabolic diseases, Hopital Necker-Enfants Malades, Paris, France.

Overgrowth can be part of various syndromes and molecular basis is known in only a few of them. In most of the cases, pathogenesis of overgrowth syndrome remains unknown, mainly when non specific overgrowth is associated with psychomotor delay. Overgrowth is a rare finding in chromosomal syndromes, but their study can give a clue for candidate genes. Here, we report on 3 patients from two unreported families with overgrowth syndrome, macrocephaly and psychomotor delay associated with trisomy 15q26 diagnosed using cytogenetic, molecular and FISH studies. Chromosome analysis of the parent showed a balanced translocation between chromosome 15q26 and the telomeric segment of chromosome 13q in the first family, and the telomeric segment of chromosome 20p in the second family. Interestingly, review of the literature showed that six other patients with terminal trisomy 15q have been reported to date, and 3 of them presented with unclassified overgrowth syndrome and psychomotor delay. The duplicated segment includes the IGF1R gene, suggesting that a dosage effect of this gene might be related to some of the clinical features observed in our patients. The present observations emphasize the importance of chromosome analysis in patients with overgrowth syndromes.

**Somatic cell hybrid analysis identifies unequivocally a case of segmental paternal isodisomy of chromosome 14.**

*K.J. Coveler<sup>1</sup>, S.P. Yang<sup>2</sup>, J.M. Milstein<sup>2</sup>, Y.Q. Wu<sup>1</sup>, C. Knox-Du Bois<sup>1</sup>, L. Beischel<sup>3</sup>, J.P. Johnson<sup>3</sup>, L.G. Shaffer<sup>1</sup>.* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) U.C. Davis Health System, Sacramento, CA; 3) Shodair Children's Hospital, Helena, MT.

Uniparental disomy of chromosome 14 (UPD 14) results in one of two distinct abnormal phenotypes depending upon the parent of origin. The phenotype of maternal disomy 14 is relatively milder than the paternal disomy 14 phenotype. This discordance is thought to result from genomic imprinting of different genes on chromosome 14. We report a case of segmental paternal isodisomy for chromosome 14 with features similar to those reported in other paternal disomy 14 cases. Molecular analysis of genomic DNA from this patient and his parents using 19 microsatellite markers spanning chromosome 14q revealed an apparent recombination event at 14q12 between markers D14S1021 and D14S121. Analysis of 12 markers between 14cen and 14q12 demonstrated biparental inheritance in this region. Data for 7 markers distal to 14q12 indicated inheritance of a single paternal allele. Hemizygosity (deletion) could not be distinguished from homozygosity (UPD). FISH with BACs RPCI-11 29E16 and RPCI-11 14N4, mapping to 14q21.1, showed hybridization to both chromosomes 14, excluding a deletion to account for the microsatellite marker results. Analysis of monochromosomal somatic cell hybrids containing either the paternal or maternal chromosome 14 revealed no deletion of the maternally inherited chromosome 14 and demonstrated unequivocally that both chromosomes contained paternal sequences from D14S121 to the telomere. Thus, the patient has paternal isodisomy for 14q12-14qter. This case likely represents a very early embryologic somatic recombination with loss of the normal cell line and the reciprocal maternal disomic cell line. A cluster of imprinted genes has been localized to 14q32. This region is fully contained within the isodisomic segment. Since the patient showed features associated with paternal disomy 14, this lends support for a single imprinted domain distal to 14q12 and excludes the proximal region of this chromosome.

**The 22q13 microdeletion : Recognise the Ring 22 chromosome phenotype to detect a new microdeletional syndrome.** *M.F. Croquette<sup>1</sup>, L. Vallee<sup>1</sup>, B. Delobel<sup>1</sup>, J.L. Bresson<sup>2</sup>, F. Fellman<sup>2</sup>.* 1) Neuropediatrics, CHRU Lille, Lille, France; 2) Cytogenetics, CHU Besancon, Besancon, France.

We detected 6 patients who had a 22q13 deletion revealed by R High Resolution banding and confirmed by FISH. Clinical evaluation of these children focused on the same physical and behavioral characteristics as 22 ring chromosome phenotype. In addition, we compared 19 new cases of ring chromosome 22. They share a common phenotype: delivery after term; at birth, the cranial circumference is slightly below the norm; facial dysmorphism is only slightly perceptible with dolicocephalia; large and/or dysplastic ears are the only frequent prominent dysmorphic feature; lymphedema with podgy hands and feet. However the whole specificity of the two syndromes lies in neurological and behavioral characteristics. Tonicity is normal at birth but hypotonia with hypertonia of limbs is increasingly frequent in the first months of life. Patients do not walk before reaching 20 months. In addition walking on tiptoes, peculiarities include very frequent falls, instability and ataxia. Neurodevelopmental delay is severe and is accompanied by mental retardation. Some first words are sometimes pronounced but suddenly the children stop speaking and many have no language. They display autistic behavior including very severe sleep disturbances in childhood. They are prone to extreme anger with tantrums. They are very unstable and exhibit obsessive forms of behavior. They are prone to eating any food, regardless of suitability, at any time without masticating. Children and adults suffer from enuresis and encopresis. Autistic traits decrease in adolescents; however some adults regress. In conclusion molecular cytogenetics demands clinical and behavioral information. Ring 22 chromosome is easy to detect but cryptic 22 q13 deletion requires FISH demonstration. According to Prasad[1] we propose that patients with a significantly delayed speech, autistic traits with minor facial dysmorphism ( dolicocephalia, large dysplastic ears ) and a normal karyotype, be screened for this deletion. This new microdeletional syndrome is certainly more frequent than literature reports. [1]PRASAD et al.(2000) Clin.Genet.103.



**A case of double mosaicism of Turner syndrome and trisomy 8.** *K.M. Christensen, P.R. Winter, F.F. Yen, G.C. Gowans, B. Angle, J.H. Hersh.* Genetics Unit, Department of Pediatrics, Weisskopf Center for the Evaluation of Children, University of Louisville, Louisville KY.

Even more rare than the presence of two aneuploidies in one cell line in the same individual, or in two cell lines with one apparently derived from the other, is the presence of two apparently unrelated aneuploid cell lines in the same individual. We report on a 12 year old female who was found on peripheral lymphocyte and fibroblast cultures to be a mosaic of 45,X and 47, XX, + 8. The proportions of Turner karyotype cell lines to Trisomy 8 karyotype cell lines were 16% to 84% in the lymphocytes and 96.5% to 3.5% in the fibroblasts. With growth hormone injections for the last year, she is at the 5th percentile of height, with a short broad neck without webbing, pectus excavatum, lateral displaced nipples, Tanner III breasts, no cardiac anomalies, pubic hair but no axillary hair, an increased carrying angle, bilateral partial 2-3 finger and toe syndactyly, and possible cross-fused ectopia of left pelvic kidney (duplication of left collecting system with blind ending right ureter). We contrast the findings in our patient with the well described findings in mosaic Turner and mosaic chromosome 8 syndromes. We compare the findings in our patient to the findings in the few previous reports of double mosaicism of Turner syndrome and trisomy 8. We discuss the most likely mechanisms underlying the mosaic cell lines.

Program Nr: 756 from the 2001 ASHG Annual Meeting

**Multiple congenital anomalies associated with *de novo* interstitial duplication of chromosome 15q11q13.** *S. Mann, N. Dorrani, D-H. Liu, C. Schanen.* Dept. Human Genetics, UCLA, Los Angeles, CA 90095.

The phenotypic consequences of chromosome 15q duplication are known to include various mild facial dysmorphisms, cognitive deficits including autism, hypotonia and seizures. We present a child with multiple congenital anomalies involving complex gastrointestinal atresias, tracheoesophageal fistula, congenital heart disease, and cloacal malformations with unilateral hydronephrosis who delivered at 30 weeks gestation. An interstitial duplication on chromosome 15 was identified by routine G-banded karyotyping, giving rise to a karyotype designation of 46, XX, dup (15) (q11-q13). FISH data with probes across the proximal long arm of chromosome 15q revealed the duplicated region included material from the Class I through Class III breakpoints. Parental karyotypes indicate that the duplication event was *de novo*. This case indicates that the phenotype associated with proximal 15q duplications may be more complex than previously anticipated. Supported by HD37470.

**FISH mapping of the Down syndrome critical region: a case with tandem duplication involving 21q22.2. R.**

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Documentation of rare cases of Down syndrome with partial trisomy of chromosome 21 has lead to the idea that there exists the Down syndrome critical region. We evaluated a patient with a duplication of 21q22 [46,XY,dup(21)(q22.1q22.3)] who had classic features of Down syndrome including flat occiput, flat facies, upslanting palpebral fissures, inner epicanthal folds, low nasal bridge, short neck, open mouth, tetralogy of Fallot, cataract, hypotonia, and mental retardation. The duplication extent of the rearranged chromosome 21 was investigated by fluorescent in-situ hybridization with BAC/PAC clones that have been mapped on the contig of chromosome 21q21~22. The BAC clone 169K17 containing D21S211 gave single signal whereas the PAC clone 307O17 containing D21S394 and the BAC clone 1P3 containing D21S15 gave two signals. Hence the proximal end of the duplicated segment is localised between D21S211 and D21S394 and the duplication spans distally beyond D21S15. The present case gives further credence to the concept that the many of the phenotypic features of Down syndrome map to 21q22.12-qter (Kornberg et al. PNAS 91:4997-5001). Considering that the proximal boundary of the duplicated segment in the present case and four cases of dup(21)(q22.1qter) reported by Kornberg et al. are all within the band 21q22.12, it is possible that genome sequence within the band might have architectural features that are prone to duplication.

**Male infant with low level mosaicism for trisomy 22 serendipitously detected by FISH.** *R.P. Ketterling<sup>1</sup>, H.C. Flynn<sup>1</sup>, G. Vockley<sup>1</sup>, D.T. Costakos<sup>2</sup>, J. Rigby<sup>2</sup>, S.M. Jalal<sup>1</sup>.* 1) Mayo Clinic/Foundation, Rochester, MN; 2) Franciscan Skemp Healthcare, Mayo Health System, La Crosse, WI.

A male infant was born at 38.5 weeks gestation to a G2P1, 35-year-old mother. The pregnancy was a product of donor artificial insemination. The prenatal history was remarkable for decreased fetal movement throughout the pregnancy and oligohydramnios noted in the third trimester. The birth weight was 1.77 kg., length was 41.9 cm. and head circumference was 29.5 cm (all well below the 5th percentile). Neonatal medical evaluation revealed multiple congenital anomalies, including a right cleft lip and palate, hypertelorism, bilateral undescended testes, hypospadias with penoscrotal inversion, absence of the corpus callosum, patent foramen ovale, moderate reflux into the left kidney, and moderately severe limb contractures. Laboratory abnormalities included hyperkalemia, hyponatremia and a single episode of hypoglycemia. Of note, the placenta demonstrated massive perivillous fibrin deposition. Neonatal aneusomy detection by fluorescence in situ hybridization (FISH) was performed and revealed a normal result for chromosomes 13, 18, 21, and XY. Conventional cytogenetic studies were performed which also revealed a normal karyotype in all 20 cells analyzed. A FISH test was performed with TUPLE1 (and ARSA internal control) to rule-out DiGeorge Syndrome (DGS). While no deletion of the DGS critical region was observed, 3 signals were observed for both the Tuple1 and ARSA probes in 32 of 200 (16%) interphase cells and in 4 of 63 (6%) metaphases. Subsequent analysis by G-banding of 33 more metaphases revealed 3 cells with trisomy 22 (total = 3 of 53 or 6%). This case represents one of the lowest reported levels for trisomy 22 mosaicism and the phenotypic features are generally consistent with those previously summarized by Crowe et al. (*AJMG* 71:406-413, 1997). These results suggest that when performing FISH tests, a careful analysis can provide unexpected information from the signal patterns observed.

**Dysmorphic features in two neonates with mosaic ring chromosome syndromes.** *P.R.K. Koduru<sup>1</sup>, S. Orner<sup>1</sup>, A. Yenamandra<sup>1</sup>, J.H. Tepperberg<sup>2</sup>, M.G. Bialer<sup>1</sup>.* 1) Dept of Pathology and Pediatrics, North Shore Univ Hosp/NYU School of Medicine, Manhasset, NY; 2) Dept of Cytogenetics, LabCorp, Research Triangle Park, NC.

Ring chromosomes with phenotypic consequences are usually detected because of a proband with developmental delay or growth retardation. We report 2 neonates with ring chromosomes who were identified mainly because of dysmorphic features. Patient #1 was seen at 29 days of age. He was the 2210g product of a 32 wk gestation pregnancy complicated by PROM for 5 wk. Mother was 36 yr old and had declined amniocentesis. Baby required some ventilatory support for a few days. Dysmorphology exam was noteworthy for bitemporal indentation, large anterior fontanelle, overfolded ear helices, cupping of the right ear, mild micrognathia and 3 transverse creases on the right palm. Chromosome analysis of peripheral blood revealed an extra small ring chromosome about 1/2 the size of a G-group chromosome in 16 of 44 metaphases analyzed. Neither parent carried the ring. FISH with a WCP-5 DNA probe painted the ring chromosome. The karyotype is described as mos 47,XY,+r[16]/46,XY[28].ish r(5)(WCP5+). Patient #2 was seen at 3 days of age. He was the 3580g product of a 39 wk gestation pregnancy born by Caesarean section after failed induction to a 23 year old mother. Baby had some initial respiratory distress which quickly resolved. Dysmorphology exam revealed right bony preaxial polydactyly, microstomia, trismus, telecanthus, hyperconvex fingernails, Sydney line and right 3rd toe held under 4th. Left choanal stenosis was noted. Chromosome analysis of peripheral blood revealed an extra ring chromosome about the size of a G-group chromosome in 6 of 20 metaphases. Neither parent carried the ring. FISH with a WCP-8 DNA probe painted the ring chromosome. The karyotype is described as mos 47,XY,+r[6]/46,XY[14].ish r(8)(WCP8+). Because ring chromosome syndromes are uncommon and their phenotypes are variable, it has been difficult to define specific features for each one. The features presumably vary based on the chromosomal material present in the ring, the percentage mosaicism in various tissues and other factors intrinsic to the host. FISH techniques are helping to define the different ring syndromes.

**Clinical/Molecular Studies of UPD14 and a Diagnostic Reversal.** *P. Papenhausen*<sup>1</sup>, *A. Wylie*<sup>2</sup>, *H. Shah*<sup>3</sup>, *J. Ranells*<sup>4</sup>, *B. Kousseff*<sup>4</sup>, *I. Gadi*<sup>1</sup>. 1) Labcorp, Inc RTP, NC; 2) Duke MC Durham, NC; 3) Nassau County MC, East Meadow, NY; 4) U. South Florida, Tampa, FL.

Recent identification of imprinting genes on the long arm of chromosome 14 have underscored the need for clinical follow-up of known cases and confirmation of imprinting status. We report the clinical reevaluation at age 9 of a girl with rare isoUPD(14)pat and a new case with isoUPD(14)mat. The DLK1 and GTL2 genes, recently shown to be normally imprinted, were studied in both cases, as well as in a clinically normal woman previously reported as isoUPD(14)mat. All three presented with 45,XX,i(14q). The first case was studied due to IUGR and polyhydramnios. Postnatal complications included prematurity, feeding and resp. difficulties requiring tracheostoma and G tube placement. She now has a 55-60 IQ with nl growth parameters, distal pectus excavatum and a mild thoracic kyphosis. Clinical comparison with other cases of UPD14pat reveals common features not reported postnatally, i.e. long philtrum, blepharophimosis, webbed neck and long fingers. Some features have resolved: hairy forehead, micrognathia, laryngomalacia, hypotonia. The female in case 2 was born at 26 wks GA. Birth wt was 1261g, length 35 cm, and HC 25.5 cm. Generalized edema, short neck, sm thorax with wide spaced hypoplastic nipples, depressed nasal bridge, sm dysmorphic ears, mild hydrocephalus, protruding philtrum and short palpebral fissures were noted. Abdominal distension from hepatomegaly and clenched long thick fingers were also seen. The infant had a PDA, was hypotonic and required intubation with ventilation support. She died of renal failure at day 51. Most features of this case have not been previously reported in maternal UPD(14) with the exception of hydrocephalus and hypotonia. Methylation analysis of the imprinted DLK1/ GTL2 region, using bisulfite treated DNA, indicated abnormal imprinting in cases 1&2 consistent with UPDpat and mat, respectively. However, normal methylation patterns were found in case 3 consistent with the normal phenotype. Expansion of the original analysis from 2 to 11 microsatellite loci revealed a non-maternal allele (father N.A.) at 4 loci consistent with biparental inheritance.

**Identifying the candidate sequence for the formation of common Robertsonian translocations.** *R. Bandyopadhyay, L.G. Shaffer.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Robertsonian translocations (Rob) are the most common rearrangements in humans. Rob(13q14q) and rob(14q21q) form predominantly during female meiosis and comprise the majority (85%) of all Robs. Previous studies have suggested that these translocations form through a specific mechanism. Our working hypothesis is that they form through homologous recombination through a common sequence shared on chromosomes 13,14, and 21. Recently a YAC based map anchored with STS-markers of the 21p region was published (Wang et al.1999). We used these STS-markers on hybrid cell lines that contained a de novo rob(14q21q), segregated from all other acrocentric chromosomes. By determining the presence or absence of the STSs, the breakpoint region was further narrowed to between two markers, D21S1276 [present] and D21S188 [deleted] on the chromosome 14/21 involved in the Robs. After narrowing the breakpoint, these two markers were used to screen a human BAC library, which identified two BACs. One BAC contains both markers and a second BAC contains only D21S188. The BACs were hybridized to six patient samples with de novo, maternally-derived rob(14q21q). FISH on the available mother's chromosomes (n=5) showed that both BACs are present on chromosomes 13, 14 and 21. The BAC containing only the marker D21S188 was deleted from all the de novo robs, while the other BAC containing both the markers was partially deleted (showed a very reduced hybridization signal, on all the rob in all cases). The presence of these BACs on chromosomes 13,14 and 21 supports the possibility that a homologous sequence shared among these chromosomes is involved in the Robertsonian translocations. Additionally, the FISH data suggest that the breakpoints are contained on one BAC between the two STS markers. Sequencing of the BACs will lead to the identification of the precise region involved in the formation of the common Robertsonian translocations.

**Asynchronous Replication of Allelic Loci in Turner Syndrome.** *R. Gal*<sup>1,3</sup>, *O. Reish*<sup>1,3</sup>, *L. Gaber*<sup>2</sup>, *A. Amiel*<sup>2,3</sup>. 1) Genetics, Assaf Harofeh Medical Center, Zerifin, Israel; 2) Genetics, Meir Medical Center, Kefar Saba, Israel; 3) Sackler School of Medicine, Tel Aviv University, Israel.

**Background :** Most allelic pairs of DNA replicate synchronously during the S phase of the cell cycle. However, some genes normally replicate asynchronously i.e. genes on the X chromosome and imprinted genes. The replication control mechanism is unknown but has been shown to be impaired in malignancies and chromosomal aberrations where the replication pattern is asynchronous. **Objective :** To determine the level of synchronization in replication timing of genes from patients with Turner syndrome (45,X0) and Turner syndrome mosaics (45,X0/46,XX). **Study Design :** The replication pattern in leukocytes obtained from patients with Turner and mosaic Turner was compared to previously karyotyped normal cells. Fluorescence in situ hybridization analysis simultaneously utilizing different probes- RB, P-53, c-myc (all red) and a probe for the X centromere (green) was performed. This enabled comparison between cells with one X (X0) and two X chromosomes (XX) in the same slide in the mosaic cases. Asynchrony was determined by the presence of one single and one set of double dots (red) in the same cell. **Results :** The asynchrony level in cells with a 45,X0 karyotype was significantly higher than in cells with a normal karyotype ( $p < 0.05$ ). Turner mosaics showed that cells with the 45,X0 genotype have a higher asynchrony level than cells with the 46,XX genotype ( $p < 0.05$ ). **Conclusions :** We assume that the loss of the X chromosome in Turner syndrome disturbs the replication and cell cycle control mechanism, leading to an asynchronous replication pattern in allelic pairs which normally replicate synchronously. The effect of this phenomenon on the Turner phenotype is yet to be determined.



**Deletion 4q21.1q23 in a newborn with hypogonadotropic hypogonadism and multiple abnormalities.** C. Guze<sup>1,3</sup>, R. Bruni<sup>1</sup>, K.S. Reddy<sup>2</sup>, R. Hassan<sup>1</sup>, D. Baker<sup>3</sup>. 1) King Drew Medical Center, Los Angeles, CA; 2) Quest Diagnostics, San Juan Capistrano, CA; 3) CSU Dominguez Hills, Carson, CA.

A male with a *de novo* deletion of 4q21.1q23 was born at 34 wks by exam, 39 wks by LMP. Weight 2130 g (50%ile), length 42 cm (10-15%ile), head circumference 34 cm (90%ile). Apgars 6 and 9. Parental chromosomes normal. He presented with significant dysmorphism: large head, wide fontanel, frontal bossing, micrognathia, short limbs, short fingers, empty scrotum, microphallus, heart murmur, significant stridor and respiratory distress requiring mechanical ventilation. Endoscopy showed laryngomalacia. EKG showed dysplastic pulmonary valve, ASD and moderate PDA. Initially diagnosed with gastroesophageal reflux disease, required G-tube at 3 months. Repeated episodes of pulmonary edema, desaturations and excessive oral secretions required oxygen supplementation and meds. No testes by ultrasound. Rarely opens his eyes and spontaneous activity limited and inappropriate. Feeding pattern inadequate; fatigue disproportionate to level of heart disease. EEG mildly abnormal, seizure meds at 2 months. MRI showed mild dilatation of frontal horns of lateral ventricles, hypoplastic vermis. He has truncal hypotonia with distal contractures of hands and feet, clinodactyly with overlapping fingers, abnormal fisting, foot drop. Renal function, calcium level, PTH, ophthalmologic exam, head CT, thyroid function all normal. Others with interstitial deletions in this region have been reported. Features common with those of our patient include developmental delay, macrocephaly, heart defects, micrognathia, general brain abnormalities, cyanosis with feeds, clinodactyly, frontal bossing, hypotonia and pulmonary incompetence. Hypogonadotropic hypogonadism (OMIM 138850) (AR) due to mutations in the gonadotropin-releasing hormone receptor gene (GNRHR) maps to 4q21.1. Males present with cryptorchidism and microphallus. Our patient showed partial hypopituitarism with hyperprolactinemia. At birth LH 1.4; FSH 1.2; testosterone 143; cortisol <0.5. He was responsive to hCG and ACTH stimulation. We propose the deletion/disruption of the GNRHR gene may be a cause for the hypogonadotropic hypogonadism in our patient.

**Prader-Willi syndrome and a deletion/duplication within the 15q11-q13 region.** *M.G. Butler, D.C. Bittel, Z. Talebizadeh.* Children's Mercy Hospital and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS), the most common genetic cause of marked obesity, is due to a paternally derived 15q11-q13 deletion, maternal disomy 15 or an imprinting mutation. There are at least three hot spots for breakage in the 15q11-q13 region [two are centromeric (BP1, BP2) and one is telomeric (BP3)]. Duplications, triplications and tetrasomy of the 15q11-q13 region have been reported with varying degrees of clinical manifestation. Generally, duplications of maternal origin have been associated with developmental delay and autistic behavior, while paternal duplications have no apparent impact on the phenotype. Herein, we describe a PWS subject with a paternally derived deletion at 15q11-q13 occurring at breakpoints BP2 and BP3 and a paternal duplication centromeric to breakpoint BP2. At 17 years of age our subject weighed 98.9 kg (> > 97th %ile) and was 149.9 cm (3rd %ile) tall. She presented with the typical features of PWS including facial appearance, hypotonia, small hands and feet, obesity in early childhood, learning and behavioral problems and skin picking. She had the typical chromosome 15q11-q13 deletion confirmed by FISH using SNRPN, D15S11, and GABRB3 probes. PCR methylation testing was diagnostic for PWS. Microsatellite analysis with PCR using 19 short tandem repeats from the 15q11-q13 region showed a paternally derived deletion of several informative loci (e.g., D15S817, D15S63, D15S210, GABRA5, D15S822) supporting breakage at breakpoints BP2 and BP3. Interestingly, she had three alleles at D15S541, D15S542 and D15S1035 centromeric to breakpoint BP2 within the 15q11-q13 region indicating a duplication of these loci. The duplication was shared by her phenotypically normal father and uncle but not by the proband's two unaffected female siblings. No other individual in the family showed the deletion. Recent reports suggest that certain repetitive DNA sequences within this region may impact on chromosome pairing. Our family further supports that a parent with a genomic duplication in this region (e.g., father) may predispose to unequal crossing over leading to a deletion event within the 15q11-q13 region and therefore PWS.

**A novel alphoid marker of the Y chromosome.** *C. Conde, S. Chheng, J. Wu, M. Santini, C.D. Kashork, S. Ware, F. Scaglia, L.G. Shaffer.* Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Many studies have concluded that alpha-satellite DNA is an integral part of the centromere and important for the normal segregation of chromosomes. However, there is an increasing number of stable human marker chromosomes have been identified that lack detectable a-satellite. Generally these marker chromosomes (called "alphoid") have lost their normal centromere through chromosomal rearrangements, leading to the formation of a new centromere (neocentromere) in a non-centromeric region on the chromosome. We describe an unusual marker of the Y chromosome. A newborn baby with ambiguous genitalia and intrauterine growth retardation was referred to our laboratory for cytogenetic studies. No dysmorphic features were noted on physical exam. Exam of the external genitalia showed a phallus that resembled a clitoris, a perineal urethral orifice, pseudovaginal labioscrotal hypospadias and hyperpigmented labioscrotal folds. Gonads could not be detected on the labioscrotal folds or inguinal canal. Pelvic ultrasound showed no defined gonads, and uterus and vagina were present with mild hydrometrocolpos. From the blood sample, an analysis of 30 G-banded cells and fluorescence in situ hybridization (FISH) analysis of 130 cells showed that the majority of cells were 45,X (85%) and the remaining cells showed a marker chromosome that was SRY positive by FISH. The centromere specific probe for the Y chromosome failed to hybridize at any site on the marker. On further evaluation, a tissue sample was requested and results revealed mosaicism for three cell lines. A monosomy X cell line was seen with conventional banding and by FISH analysis in 85% of cells. Two additional cell lines were noted in low level by conventional banding and seen at higher rates in FISH analysis. The first, seen in 9% of cells, contained an acentric marker that hybridized with one copy of SRY. The second cell line, seen in approximately 6% of cells, contained an acentric marker that hybridized with two copies of SRY. These results have been interpreted as an unstable marker of the distal short arm of the Y chromosome and this marker likely represents the first case of neocentromere formation in Yp.

**The shape and DNA mediated banding pattern of chromosomes in interphase are similar to metaphase**

**chromosomes.** *U. Claussen*<sup>1</sup>, *J. Lemke*<sup>1</sup>, *J. Claussen*<sup>1</sup>, *I. Chudoba*<sup>2</sup>, *V. Trifonov*<sup>3</sup>, *N. Rubtsov*<sup>3</sup>, *A. Heller*<sup>1</sup>, *H. Starke*<sup>1</sup>, *K. Sperling*<sup>4</sup>, *T. Liehr*<sup>1</sup>. 1) Inst Human Gen & Anthropology, Friedrich-Schiller-Univ, Jena, Germany; 2) MetaSystems GmbH, Robert-Bosch-Str. 6, D-68804 Altlussheim, Germany; 3) Institute of Cytology and Genetics SB RAS, 630090 Novosibirsk, Russia; 4) Institute of Human Genetics, Humboldt-University, Augustenburger Platz 1, D-13353 Berlin, Germany.

Interphase chromosomes analysed with currently available techniques do not present any recognizable structures such as bands, centromeres, telomeres, or specific shapes. Microirradiation experiments and molecular cytogenetic investigations with whole chromosome paints and region specific microdissection probes have confirmed a territorial organization of chromosomes in interphase nuclei. Until now, however, their structure is not well understood. Using the high-resolution DNA-based multicolour banding technique (MCB), we have generated a banding pattern and have determined the length of human chromosome 5 in lymphocyte interphase nuclei, and in nuclei of HeLa cells arrested at different phases of the cell cycle. Chromosome 5 in interphase nuclei is bent and folded and shows an MCB pattern similar to that of metaphase chromosome 5 at all stages of the cell cycle. The length of the chromosome axis is comparable to that of a metaphase chromosome at a 700-band resolution. Therefore, the concept of chromosome condensation during mitosis has to be reassessed. Interphase chromosome banding was successfully used to identify chromosome aberrations and opens new fields in cytogenetic analysis.

**45,X due to translocation of the Y chromosome onto the short arm of chromosome 15 in males of a 3-generation family and evidence for somatic instability.** *P.M. Gopinath<sup>1</sup>, C.S. Nagarajappa<sup>1</sup>, N. Chandra<sup>1</sup>, B. Palaniappan<sup>1</sup>, R. Wimmer<sup>2</sup>, I. Hansmann<sup>3</sup>.* 1) Dept. of Genetics, Madras University, Chennai, India; 2) Institut fuer Humangenetik, Freiburg, Germany; 3) Institut fuer Humangenetik und Medizinische Biologie, Halle/Saale, Germany.

45,X results in a female phenotype Turner syndrome but is rarely associated with maleness. In some of these males Y chromosomal material is translocated onto the X or to an autosome. Most often such translocations are de novo events associated at least with infertility or subfertility. We report on a 3-generation family with four 45,X males in which a Y/15 translocation segregated from grandfather to father and to his 2 sons without any apparent effect on fertility and/or phenotype. The propositus, a 11-year-old male, was referred for karyotyping because of small testis and penis and non-erection of phallus. The right testis was only partially descended. Chromosome analysis revealed 45,X including a Y/15 translocation with a prominent heterochromatic block proximal on the short arm creating a pseudodicentric der(15) with apparent inactivation of Y centromere. Breakpoints are tentatively assigned to distal Yq12 and 15p11.2. His younger brother, his father and grandfather also were found to possess 45,X and the same translocation. Chromosome Y paint completely labeled the short arm of der(15). FISH using probes for PAR1 (e.g. SHOX) and PAR2 (e.g. SYBL1) revealed signals at the distal short arm of the X and the der(15) for PAR1 and at the distal Xq for PAR2. No signal for PAR2 was observed within the short arm of der(15) indicating loss of PAR2. Loss of PAR2 has only rarely been observed, e.g. in cases of satellited Y chromosomes (Kuhl H. et al. 2001) and it could be speculated that conservation of PAR2 might be required for the stability/integrity of the Y chromosome. We do have evidence from our propositus with PAR2 deletion that the Y;15 translocation might not be stable mitotically, either in vivo or in vitro. Approx. 20% of cultured lymphocyte metaphases did show an additional Y chromosome besides the der(15). Also by interphase FISH with Y specific probe approx. 13% of nuclei did show two separate Y signals.

**Delineation of phenotypically normal chromosomal duplications: Implications for detection and involvement of repetitive DNA.** *M.D. Graf, E.E. Eichler, A.B. Zinn, S. Schwartz.* Ctr for Human Genetics and Dept of Genetics, Case Western Reserve Univ and Univ Hospitals of Cleveland.

Duplication of chromosomal material can occur as a consequence of various structural rearrangements. Most commonly, duplications result from meiotic malsegregation of balanced chromosomal rearrangements (translocations, inversions, and insertions) from a phenotypically normal carrier. However, a proportion are due to de novo intrachromosomal (i.e. tandem) or interchromosomal rearrangements. Additionally, some intrachromosomal duplications are transmitted directly from a phenotypically normal parent. Over the past 10 years, to better understand the phenotype-karyotype implications of different duplications, we have studied over 100 de novo and directly transmitted duplications (not including those that have been inherited due to malsegregation of parental rearrangements). We have utilized high-resolution chromosomal analysis and fluorescence in situ hybridization, mostly with single copy probes, to characterize the duplicated material. Our studies have revealed that 10-15% of these duplications (mostly intrachromosomal) are directly inherited from a phenotypically normal parent with varying phenotypic consequences. In addition, 5% of the de novo duplications had no associated abnormal phenotype. To better understand these cases, we have developed BAC probes and have shown that most cases involve the duplication of repetitive material.

Based on the findings from these studies we have gained additional insights concerning the implication of duplicated material and can conclude the following: (1) a larger than expected number of cases of tandem duplications were directly inherited, while only one interchromosomal duplication was; (2) several of the duplications were not associated with phenotypic abnormalities, suggesting that the duplication consists of only repetitive and/or paralogous DNA; and (3) by utilizing information from the human genome project, we have developed several probes for the different duplications to confirm that the duplications often involve repetitive, rather than euchromatic, material.

**Subtelomeric assay as a genetic screening tool for MR of unknown etiology.** *A.J. Dawson<sup>1,2,3</sup>, S. Putnam<sup>3</sup>, D. Riordan<sup>3</sup>, A. Mhanni<sup>1,2</sup>, S. Kovnats<sup>1</sup>, S. Seshia<sup>4</sup>, C. Prasad<sup>1,2</sup>.* 1) Department of Pediatrics and Child Health, University of Manitoba; 2) Department of Biochemistry and Medical Genetics, University of Manitoba; 3) Cytogenetics Laboratory, Division of Laboratory Medicine and Pathology, HSC, Winnipeg, Manitoba; 4) Manitoba Clinic, Winnipeg, Manitoba.

The telomeres of human chromosomes are gene rich. Chromosome telomere rearrangements occur with a frequency of 7-10% in children with mild to moderate MR and approximately one half of cases are familial. Clinical investigation for subtelomeric rearrangements is now prompted by FISH analysis using specific DNA probes from all relevant chromosome ends. In our study, 20 children were selected for subtelomeric assay using the Chromophore Multiprobe-T Cytocell device. Inclusion criteria were developmental delay or MR, a normal 550 band G-banded karyotype, FRAXA negative, and at least one other clinical criterion. Exclusion criteria included an identified genetic, environmental, or teratogenic diagnosis. Parents had to consent to cytogenetic subtelomere analysis if necessary. Of the 20 patients analyzed, one (5%) was found to have an unbalanced female karyotype with a derivative chromosome 9 resulting from a translocation between the telomeres of the long arms of chromosomes 5 and 9: 46,XX.ish der(9)t(5;9)(q35.3;q34.3) (D5S2097-,D9S2168+;D5S2097+, D9S2168-)pat. The father was a balanced de novo carrier for this translocation as paternal grandparents had normal karyotypes. The patient's brother was also a balanced carrier. The proband's mother had a history of two miscarriages. At nine months of age, the patient had a weight of 7.6kg (10th-25th%ile), height of 68.5cm (25th-50th%ile) and an OFC of 39.9cm (2nd%ile). She had significant midface hypoplasia with a high, sloped forehead and significant microcephaly. The anterior fontanel was patent and there was no ridging of any cranial sutures. Her DQ was 40. The cranial MRI showed marked deep white matter changes in the posterior half of the brain with generalized atrophy. Our findings support the view that screening for subtelomeric rearrangements is a significant and cost-effective way of making diagnoses in children with unexplained mental retardation.

**Distribution of exchanges in chromosome 15 nondisjunction.** *J.L. Gair, B.D. Kuchinka, W.P. Robinson.*

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Nondisjunction (ND) leading to trisomy and other aneuploidies contributes significantly to reproductive difficulties and human genetic disease. Understanding factors affecting ND in humans is necessary if we are ever to develop therapies to reduce its occurrence. Studies of recombination in meiotic ND leading to trisomy 21, suggest that both alterations in the amount and the distribution of recombination along the chromosome are associated with ND. Previous studies of ND for chromosome 15 also show a reduction in recombination in meiosis I (MI) errors. To investigate recombination and chromosome 15 ND further, molecular marker typings from 157 cases of trisomy 15 or UPD15 were used to divide chromosome 15 into 14 intervals. The amount and distribution of crossovers in each interval was then compared to CEPH controls (public and own data). A comparison of genetic and physical maps was also made to test if recombination correlates closer with physical distance with ND, suggesting relaxation of chromatin influences on recombination. As sequence is not complete for this chromosome, physical distance was estimated from radiation hybrid maps. Comparisons of MI and meiosis II (MII) errors with CEPH control females, shows that there does not appear to be an alteration in the distribution of recombination along chromosome 15 in any but the most proximal region from D15S541 to D15S122 (with estimates of 0.96 cM, 10.8cM and 15.59 cM for MI errors, CEPH control females and MII errors, respectively). The distance between double crossovers was also estimated to determine if interference patterns might be altered in ND. Of 56 double crossovers identified among MI errors, most (59-86%) occurred within 50cM. Of these, 8 definitely and 26 possibly, occurred over less than 30cM genetic distance. In contrast, double crossovers within such a short distance were extremely rare among 50 double crossovers identified in CEPH controls. The shortest distance of double crossover was 8-16cM among MI errors and 11-17 cM among CEPH female controls. Further refinement of crossovers and analysis will be necessary to determine if relaxed interference is associated with ND.



**The Importance of Chromosome Analysis for Assessing the Recurrence Risk in Cases of Anencephaly.** *G.S. Frohlich, D.B. Rogers, M. Thangavelu.* Genzyme Genetics, Orange, CA.

The prenatal diagnosis of anencephaly commonly leads to a recurrence risk based on multifactorial inheritance. However, if a chromosome abnormality is detected, the risk for recurrence can lead to vastly different genetic counseling for the patient. The incidence of chromosome abnormalities in anencephalic fetuses is, at best, poorly documented in the literature. We have reviewed the Genzyme Genetics database of cytogenetic analyses performed (n = 292) on pregnancies affected with anencephaly. The indications for analysis and cytogenetic abnormalities were reviewed. Our review revealed chromosome abnormalities consisting of numerical (n = 13, 4.4%) and structural (n = 4, 1.3%) defects, accounting for greater than 5% of the total number of anencephaly cases. Two cases with structural abnormalities had partial duplication of chromosome 2. The recurrence risk following a single occurrence of anencephaly is based on the overall incidence of neural tube defects in a particular population. This may vary from 1-2% in the United States to as high as 5% in other areas of the world where the NTD incidence is much higher. Genetic counseling following the diagnosis of a chromosome abnormality can, in many instances, be more precise and could signal the existence of significant risks for the patient and possibly other members of his/her family. Structural chromosome abnormalities can, depending on whether they are de novo or familial, lead to genetic counseling recommendations varying from minimal risk for recurrence to the offering of prenatal diagnosis to multiple family members. It is our conclusion that, in cases of anencephaly discovered on prenatal ultrasonography, chromosome analysis be offered in order to identify familial rearrangements and to provide the most appropriate genetic counseling for the patient.

**A prospective, double-blind study comparing results of G-banding and SKY methodologies in 89 neonates referred for cytogenetic testing.** *A. Anguiano, V. Sulcova, A. Sbeiti, K. Hoang, S. Wang, A. Fraser, M. Ayad, K. Reddy, B. White.* Cytogenetics Dept, Quest Diag, Nichols Inst, San Juan Capist, CA.

Spectral karyotyping (SKY) has proven very useful for detecting various chromosomal rearrangements not revealed by conventional banding methods. To further assess the utility of SKY, we tested 89 neonates in a prospective, double-blind study that utilized both conventional cytogenetics and SKY methodologies. Cases with common numerical abnormalities were excluded via a preliminary assessment of five G-banded metaphases derived from a 24-hour culture. The non-aneuploid cases were then evaluated by SKY in parallel with G-banding studies. In each case, at least 20 G-banded metaphases were examined during conventional cytogenetic analysis, and a minimum of 3 metaphases were studied using SKY. Emphasis was placed on examination of telomeric regions. Conventional G-banding identified six abnormal cases. SKY revealed the same 6 cases and one additional abnormality, a trisomy 8 mosaicism. On the other hand, G-banding defined a mosaic with two abnormal and one normal cell line, while SKY detected the normal cell line and only the more prevalent abnormal cell line, missing the second abnormal cell line observed in 25% of G-banded metaphases. Thus, SKY did not demonstrate significantly improved sensitivity relative to G-banding. While it was useful to detect the one mosaic case missed by G-banding, cryptic imbalances such as telomeric-region rearrangements or insertions were not revealed by SKY. Possibly larger series will detect such abnormalities. At this point it does not seem justified to routinely recommend SKY for further investigation of phenotypically abnormal neonates showing a normal G-banded karyotype.

**A contiguous gene syndrome in an infant with both cleidocranial dysplasia and CHAR syndrome due to deletion 6p21.2-p11.2.** *Y.S. Choy<sup>1</sup>, S.K Tan<sup>2</sup>, A. Othman<sup>2</sup>.* 1) Cons Gen/Neurologist/Ped, Kuala Lumpur Hosp/Ped Inst, Kuala Lumpur, Malaysia; 2) Cytogenetic Department, Kuala Lumpur Hospital.

The coexistence of two rare Mendelian disorders in an individual is uncommon but can be explained by the deletion of a sequence of adjacent genes. Cleidocranial dysplasia is an autosomal dominant skeletal dysplasia due to mutations in the RUN-related transcription factor 2 gene located at 6p21. An adjacent gene at 6p12, TFAP2B gene, causes CHAR syndrome in a dominant negative manner. Most of the other genes with known functions in the region 6p21.2-p11.2 are inherited in an autosomal recessive manner except for RP7 which causes adult onset retinitis pigmentosa and HMGIIY which is responsible for lipoma. Therefore, haploinsufficiency of the region 6p21.2-p11.2 would result in a contiguous gene syndrome with these disorders. We described a 16-month old Malay boy born to a 28-year-old G1P1 mother and a non-consanguineous father. Multiple birth defects and dysmorphism were noted at birth including widely separated sutures and widely open anterior fontanel with parietal bossing, hypertelorism, flat short nose, long philtrum, thick lips, micronagthia and high arched palate, narrow thorax, short digits, bilateral clinodactyly. Both the clavicles were absent. He had a moderate sized patent ductus arteriosus requiring anti-failure therapy. Karyotype analysis revealed a interstitial deletion of 6p21.1-p11.2. Parental karyotypes were both normal. Subsequently, he had recurrent respiratory distress needed bronchodilator therapy. Bilateral mild conductive deafness was detected. Beside a delay in fontanel closure, there was delay in eruption of deciduous teeth with enamel hypoplasia and malalignment consistent with the natural history of cleidocranial dysplasia. His facies on the other hand became more reminiscent of CHAR syndrome where he has duck-bill like lips associated with flat face and broad flattened nasal tip. renal ultrasound and MRI of the brain were normal, He has a relatively normal milestone thus far. His eyes are normal now but being followed up closely. This case illustrated the correlation of cytogenetic abnormality and phenotype of the deleted region 6p21.2-p11.2.

**Detection of a submicroscopic deletion 1p36.3 in a patient with mosaicism for trisomy 9p resulting from der(1)t(1;9)(p36.3;p13) using a subtelomeric FISH probe.** *N.J. Carpenter<sup>1</sup>, X. Yang<sup>2</sup>, B. Moghaddam<sup>1</sup>, M.E. Floyd<sup>1</sup>, K.S. Reddy<sup>2</sup>.* 1) HA Chapman Institute of Medical Genetics, Tulsa, OK; 2) Quest Diagnostics, Inc., San Juan Capistrano, CA.

Mosaicism involving an apparently normal cell line and an unbalanced autosomal structural aberration that is not a ring or a supernumerary marker chromosome is rare. This report describes an infant with mosaicism for an unbalanced translocation for whom the apparently normal cell line was found to have a submicroscopic deletion. Prenatal diagnosis was performed due to fetal hydrocephalus and IUGR and a 46,XX karyotype was observed. At birth, the infant had patent ductus arteriosus, generalized hypotonia, bilateral optic nerve hypoplasia, clitoromegaly and pes calcaneovalgus. At 6 months of age, she has dysmorphic facies, bitemporal depression, low-set ears, inverted nipples, and significant developmental delay. Cytogenetic and FISH studies of peripheral blood showed a mosaic karyotype, 46,XX,der(1)t(1;9)(p36.3;p13).ish der(1)(wcp9+)/46,XX, resulting in trisomy 9p in 88% of the cells. A retrospective study of the prenatal sample revealed only 4% of the cells had the der(1). Surprisingly, FISH analysis of the blood with a subtelomeric probe for 1p detected a deletion of 1p36.3 in the apparently normal cell line as well as in the der(1) cell line. The mother has a 46,XX karyotype and FISH study is in progress. The father is unavailable. The infant has some features of the del(1p) syndrome, some of the dup(9p) syndrome, and some which are common to both. It is postulated that the del(1p) was present in the zygote as a result of a de novo deletion or a familial cryptic translocation. Exchange of chromatids of chromosomes 1 and 9 during mitosis, loss of one of the translocated chromatids, and segregation resulted in the del(1p) and the der(1)t(1;9) cell lines. The deletion of the 1p telomere may have increased the likelihood of a mitotic exchange involving this chromosome region. The finding of the del(1)(p36.3) in the apparently normal cell line means that the possibility of a familial translocation must be investigated to assess the risk for future pregnancies and for other family members.

**Deletion of distal Yp chromosome resulting from a dicentric chromosome(Y;22) in a child with short stature and no dyschondrosteosis.** C. Borie<sup>1</sup>, J. Leger<sup>2</sup>, O. Dupuy<sup>1</sup>, N. Bondeux<sup>1</sup>, C. Pignal<sup>1</sup>, C. Dupont<sup>1</sup>, P. Czernichow<sup>2</sup>, P. Eydoux<sup>1</sup>. 1) Biologie du Developpement; 2) Endocrinologie, Hospital Robert Debre, Paris, France.

We report a 4 year-old child with short stature, and a dicentric chromosome with a deletion of the distal part of chromosome Yp. The parents were not consanguineous, and did not have any relevant history of genetic disease. The pregnancy was uneventful, until intra-uterine growth retardation was noted. Prenatal karyotyping showed a de novo(Y;22) translocation. No structural fetal abnormality was shown at ultrasound examination, and the pregnancy went to term. A growth retarded boy with an otherwise normal physical examination was delivered at 39 weeks. At age 4, the child had short stature (-3SD) without mental retardation. Radiological examination of the wrist was normal. A blood karyotype confirmed the chromosomal rearrangement seen on amniotic fluid cells. C-banding showed a dicentric chromosome, and FISH with centromeric probes confirmed the presence of both chromosome Y and 22 centromeres on the derivative chromosome. FISH using a TUPLE1 probe did not show any rearrangement at 22q11 or at the ARSA locus. Using a subtelomeric probe, a deletion of the distal Yp region was shown. The karyotype was thus 45,X,der(Y;22)(p11;q11)del(Y)(p11p11). We hypothesise that the chromosomal rearrangement of this patient resulted in the deletion of the distal part of the pseudoautosomal region PAR1, including SHOX but not SRY. Haploinsufficiency of the former gene may result in Leri-Weill dyschondrosteosis, which did not appear to be an obvious diagnosis in this young patient. Further studies are pending to demonstrate the extent of the Yp deletion.

**Non-convulsive status epilepticus and ring chromosome 20: a specific cytogenetics syndrome.** *O. Dupuy<sup>1</sup>, C. Borie<sup>1</sup>, A. Arzimanoglou<sup>2</sup>, N. Collot<sup>1</sup>, A. Thaly<sup>1</sup>, M. Vitu<sup>1</sup>, P. Evrard<sup>2</sup>, P. Eydoux<sup>1</sup>.* 1) Biologie du Developpement; 2) Epilepsy Program, Child Neurology and Metabolic Diseases Dpt., University Hospital Robert Debre, Paris, France.

Our patient, a 14 years old girl, was born after an uneventful pregnancy, from non consanguineous parents with no relevant history of a genetic disease. Physical examination was normal at birth and further development was reported as normal. The first paroxysmal episodes were observed at age 9. They occurred mostly on awakening, and consisted of a prolonged confusional state, slowness of response, wandering, oro-motor automatism and redness of the face. The child could recall the events during periods of mild confusion and exhibited fear, often related to hallucinations involving animals such as spiders or reptiles. Episodes were markedly long, lasting from 15-20 minutes up to one hour. Ictal EEGs showed long-lasting high-voltage slow waves with occasional spikes, with a frontal predominance. The blood karyotype was performed because of this very peculiar type of epilepsy, and showed a ring chromosome 20 in 7 out of 18 cells. One double ring was observed. FISH, using subtelomeric probes, showed the presence of both 20p and 20q signals, thus did not reveal any distal deletion of the chromosome 20 subtelomeric regions, as would be expected in a ring chromosome resulting in phenotypical anomalies. Epilepsy characterized by prolonged episodes of non-convulsive status and associated with spider or reptilian hallucinations is a very specific syndrome and warrants karyotyping. The mechanism resulting in this syndrome is not well understood, since no specific deletion seems to be associated with the ring chromosome. Hypotheses include a deletion very close to the telomere, mosaicism with a heterogeneous population (double ring chromosome, broken ring, chromosome 20 deletion), or a position effect around the subtelomeric region resulting in an abnormal inactivation or activation of gene(s) within this region.

**Genetic study on 231 women affected by premature ovarian failure (POF).** *L. Dalprá<sup>1</sup>, C. Porta<sup>2</sup>, E. Martinoli<sup>2</sup>, A. Patrizi<sup>2</sup>, E. Sala<sup>3</sup>, N. Villa<sup>3</sup>, MG. Tibiletti<sup>4</sup>, M. Taborelli<sup>4</sup>, B. Dossena<sup>4</sup>, E. Ginelli<sup>2</sup>, A. Marozzi<sup>2</sup>.* 1) Dept Experimental and Environmental Medicine and Medical Biotechnology, Univ.Milan-Bicocca, Italy; 2) Dept Biology and Genetics for Medical Sciences, Univ Milan, Italy; 3) Genetic Lab, H.S.Gerardo, Monza, Italy; 4) Dept Clinical and Biological Sciences, Univ. Insubria, Varese, Italy.

The absence of germ cell is associated with a complete ovarian failure, while the decrease number is more likely associated with partial ovarian failure or secondary amenorrhoea. Primary and secondary amenorrhoea are highly heterogeneous and their aetiology is still unknown in most cases, and these conditions may be considered as the different expression of the same disorder. A chromosomal and genetic role is universally recognised in the pathogenesis, but the frequencies and the types of chromosome aberrations and gene mutations are to be determined. A systematic genetic study on 231 (21 primary and 210 secondary amenorrhoea) women affected by POF was performed, including chromosome analysis and FRAXA premutation screening. The inclusion criteria for the study were menopause before of age 40, FSH values > 40 IU/l and normal phenotype. The overall percentage of chromosome anomalies was 7%, including 3 pure autosomal abnormalities, 3 Xq deletions, 1 Xp deletion, 1 psudic(Xp) (the only observed aberration in primary amenorrhoea), 2 translocations involving chromosome X and autosome (1 balanced active and 2 unbalanced completely inactivated), 1 translocation (Xp;Xq), 1 XXX and 4 numerical and structural mosaicisms. For cases in which a single cell with 45,X and/or 47,XXX karyotype were detected, further analysis by means of interphase FISH were carried out. The observation of single or triple signals confirmed the hypothesis that the mosaic situations can be an underestimated event. All these data indicate that both monosomy and triple dosage of X regions are responsible of ovarian failure. Moreover, FRAXA premutation screening on 179 POF women with normal karyotype indicates a premutation status in 9 patients (5%). In conclusion, FRAXA premutation testing and chromosomal analysis must be done in order to identify the cause of ovarian failure.

**Multiple copies of SHOX does not overcompensate for the loss of Yq in a male with short stature and iso Yp.** *L.E. Evans<sup>1</sup>, V. Shashi<sup>1</sup>, A.R. Turner<sup>1</sup>, R. Schwartz<sup>2</sup>, M.J. Pettenati<sup>1</sup>.* 1) Department of Pediatrics/Section on Medical Genetics, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Department of Pediatrics, Wake Forest University School of Medicine, Winston-Salem, NC.

Routine cytogenetic analysis was performed on a 3 2/12 year old male, presenting with short stature, delayed bone age, developmental delay and small testicles. His karyotype revealed a structurally normal X chromosome and an apparent isodicentric Yp chromosome rearrangement. Molecular cytogenetic analysis confirmed that this was an isodicentric Yp chromosome lacking centromeric material and having two copies of the SHOX (Xp22.3) and SRY (Yp11.3) gene on either end of the isochromosome [46,X,rec(Y)i(Y)(p11.1)t(X;Y) (p22.33;p11.32)]. His X chromosome had a single SHOX gene on the Xp. Thus, he was trisomic for SHOX. Parental chromosomes were normal. This rare recombination probably occurred in two stages. The first event most likely was a paternal meiosis I error, involving the translocation of a region on Xp including the SHOX gene to the distal end of Yp, followed by a second event, when the Y chromosome missegregated, resulting in an isochromosome.

The pseudoautosomal short stature homeobox-containing gene (SHOX) is believed to exert a dosage effect in sex chromosome aberrations. Haploinsufficiency of the SHOX gene has been shown to cause short stature and overdosage of the SHOX gene has been implicated in tall stature among females. Overdosage of the SHOX gene specifically in males has not been described. Although the presented male has 3 copies of the SHOX gene, he demonstrates short stature. This phenotype may be best explained by the lack of Yq, which has been associated with short stature and infertility in males. Thus, we suggest that the deletion of Yq is dominant for stature over the overdosage of SHOX.



**Partial trisomy 11q due to a  $\text{ins}(22;11)(q13.3;q14.2q23.3)\text{pat}$ .** A. Cervantes<sup>1</sup>, R. Guevara-Yáñez<sup>2</sup>, J.T. Granados<sup>1</sup>, E. Gálvez<sup>1</sup>, V. Madrid<sup>1</sup>, N. Téllez<sup>1</sup>, M. López<sup>3</sup>, S. Kofman<sup>1</sup>. 1) Genética, Hospital General México/Facultad Medicina, UNAM; 2) UIM Genética Humana, H Pediatría CMN SXXI, IMSS; 3) S Biológicos, UAM-X, México D.F.

Most cases of partial trisomy 11q involve a  $\text{t}(11;22)(q23;q11)$  with a 3:1 meiotic segregation and includes clinical manifestations of trisomy 22. In some cases, trisomy 11q can be linked to a partial monosomy of another chromosome due to adjacent I segregation of a parental reciprocal translocation. Insertions or de novo duplications are found rarely. The breakpoints involved vary from 11q12.1 to 11q23.2 and the severity of manifestations are related to the extent of the implicated segment. The major diagnostic criteria includes development delay, growth retardation, and characteristic facies.

A five-year-old girl with moderate psychomotor retardation and language anomalies is reported. She had low-set ears, divergent strabismus, short and wide nose, anteverted nares, prominent upper lip, high palate, retrognathia, short neck, funnel chest, fifth toe clinodactyly and cutaneous syndactyly 2-3 of toes. Her karyotype with GTG banding revealed a  $46,XX,-22,+der(22)$  complement. The karyotype of her mother was normal and the father and grandfather's karyotypes were  $46,XY,\text{ins}(22;11)(q13.3;q14.2q23.3)$ . Fluorescent in situ hybridization (FISH) was performed in the patient and her father. A chromosome painting cocktail (ONCOR) for chromosome 22 painted only normal and derivative chromosomes 22; telomeric probes for 11p, 11q and 22q (CYTOCELL) were used to confirm the insertion. We discuss the mechanism of formation of der (22) and compare clinical data with different cases of pure trisomy 11q described in the literature.

**Prenatal diagnosis of trisomy 1p36.3 and monosomy10q26.** *S.G. Adhvaryu<sup>1</sup>, L.G. Shaffer<sup>2</sup>, R.C. Lewandowski<sup>1</sup>.* 1) Center for Genetic Services, Corpus Christi, TX 78412; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030.

Amniotic fluid sample from 25 years old (G2P1) was received for chromosome analysis because of elevated risk for fetal Down syndrome based on abnormal maternal serum screen. Chromosome analysis revealed the fetal karyotype as an abnormal female: 46,XX,?del(10)(q26). Parental chromosome analysis showed the father to be a carrier of a balanced translocation involving chromosomes 1 and 10, with the breakpoints at p36.3 and q26.1, respectively. Fluorescence in-situ hybridization (FISH) studies with telomere-region specific probes were pursued for confirmation of the breakpoints. A female infant was born at 32 weeks gestation with a birth weight of 1610 gm. Other clinical findings at birth were respiratory distress syndrome, bilateral clubfeet, abnormal eye movement and low platelets. A detailed physical examination on 21st day revealed a child with dolichocephaly with slight frontal bossing, bilateral epicanthus, blepharophimosis with a concurrent telecanthus, anteverted nares, micrognathia and abnormal ears with folding of the helix bilaterally. FISH studies are an important, integral part of the cytogenetic laboratory in identification of subtle chromosome rearrangements. Few cases of trisomy for the terminal short arm of chromosome 1 (1p36.3) exist in the literature. This infants physical findings are compounded by the double segmental imbalance of concomitant monosomy for the distal long arm of chromosome 10 (10q26). Given the identification of a parental translocation, this family is at significant risk of the same or the reciprocal imbalance in future pregnancies.

**Subtelomeric Chromosome Abnormalities in South Carolina.** *B.R. DuPont, J.S. Collins, J.G. Archie, R.S. Saul, C.E. Schwartz, R.E. Stevenson.* Greenwood Genetics Center, Greenwood, SC.

Fluorescence in situ hybridization (FISH) analysis of chromosome subtelomeric areas has enhanced the search for the underlying etiology in patients with idiopathic mental retardation. Previous studies have shown between 4% and 23% of children with moderate to severe retardation have a subtle chromosome abnormality using subtelomeric FISH probes. The largest study (Knight et al., 1999) found approximately 7% of patients had an unbalanced telomere rearrangement. A subsequent analysis of their data [29 affected, 110 controls] demonstrated that the prenatal onset of growth retardation and a positive family history for mental retardation are good indicators for subtelomeric analysis (STA) (deVries et al., 2001).

The Greenwood Genetic Center Cytogenetics Laboratory initiated STA in 2000. To date, we have analyzed 43 people with developmental delay. We found subtelomeric abnormalities in 6 patients (3M, 3F), 4 Caucasian, 1 African-American, and 1 Indian. They ranged in age at testing from 2 months to 56 years, with 4 below the age of 8 and 2 above the age of 50; birth weights fell into normal ranges. Clinical features and abnormal test results that occurred in more than one of these individuals included facial dysmorphism (6/6), skeletal abnormalities (2/5), CNS abnormalities (3/5), cardiac abnormalities (3/5), vision abnormalities (2/5), cranial image abnormalities (3/4), and other abnormalities (3/5). The chromosome aberrations included der(4)t(4;11), 8p-, 8p+, 9p-, der(13)t(5;13), and der(14)t(7;14). Three of these rearrangements were familial in origin, one was de novo, and results on the others were pending.

In reviewing the causes of mental retardation in 7600 persons in South Carolina, we found 2513 with moderate to severe MR with unknown causation and an additional 324 with MR and a family history. If these two groups are combined, we might expect a retrospective analysis to find as many as 210 STA abnormalities using a 7.4% estimate.

**Pallister-Killian Syndrome Due to a Novel Chromosome Abnormality: A Case of 12p Intrachromosomal**

**Triplication.** *H.M. Bedford*<sup>1</sup>, *S. Unger*<sup>1</sup>, *M. Nowaczyk*<sup>2</sup>, *I. Teshima*<sup>3</sup>. 1) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Clinical Genetics Services, Hamilton Health Sciences Corporation, Hamilton, ON, Canada; 3) Molecular Diagnostics, Department of Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, ON, Canada.

Pallister-Killian Syndrome (PKS) is a rare sporadic condition characterized by multiple congenital anomalies, mental retardation and tissue-limited mosaicism for isochromosome 12p (i(12p)). Children with PKS surviving beyond the newborn period typically present with seizures, severe developmental delay, hypo/hyperpigmented streaks of skin, disproportionate shortening of the limbs and characteristic craniofacial features. The mechanism(s) of formation and parental origin of the isochromosome are not well understood. The i(12p) is usually present in fibroblasts but is rarely seen in leukocytes. Selection against the i(12p) chromosome in leukocytes or anaphase lag may explain the lack of abnormal cells in blood. In the majority of cases in which parent of origin was studied, the i(12p) was maternal in origin.

We present an 18-month-old boy with characteristic features of PKS due to a novel chromosome 12 abnormality. Peripheral blood karyotype was normal (46,XY), however chromosome analysis of cultured fibroblasts demonstrated mosaicism for a 12p+ chromosome. This 12p+ chromosome was further characterized by C-banding and molecular cytogenetic techniques including whole chromosome painting and fluorescence in-situ hybridization (FISH) analysis to determine the chromosomal origin of the additional material and to define the breakpoints. The resulting final karyotype was 46,XY, trp(12)(p11.2p13)[113]/46,XY[2]. This is the first case of PKS due to an intrachromosomal triplication of 12p. In addition, this is the first case of an intrachromosomal triplication involving chromosome 12. Intrachromosomal triplications involving chromosomes 2, 5, 7, 9 and 15 have been previously reported. The mechanism(s) of intrachromosomal triplications has been debated. This case provides evidence for a postzygotic event in the generation of some intrachromosomal triplications.

**A mosaic ring chromosome 4 in a fetus with Wolf-Hirschhorn phenotype - breakpoint mapping and histopathology.** *S.U. Endeke<sup>1</sup>, A. Kocks<sup>1</sup>, B. Schroeder<sup>1</sup>, R. Heller<sup>1</sup>, M. Makrigeorgi-Butera<sup>2</sup>, C. Staedtler<sup>3</sup>, H.J. Schaefer<sup>3</sup>, A. Winterpacht<sup>1</sup>.* 1) Inst. of Human Genetics, Hamburg, Germany; 2) Dept. of Neuropathology, University of Hamburg, Germany; 3) Inst. of Pathology, University of Hamburg, Germany.

The molecular basis of Wolf-Hirschhorn syndrom (WHS), also deletion 4p syndrome, has not yet been elucidated. However, based on genotype-phenotype correlation studies and by molecular analysis of chromosomal breakpoints on chromosome 4p, the critical region has been narrowed to an interval of 165 kb between D4S43 and FGFR3. Several candidate genes are being tested for their role in WHS by us and others using cloning techniques and mouse knock-out models. The study of dysmorphological and histopathological features in affected human fetuses with well characterized chromosome 4p deletions is an additional strategy to gain insight into the developmental pathology of WHS. We present the case of an aborted fetus (21st gestational week) with Wolf-Hirschhorn phenotype: pronounced growth deficiency, complex cardiac defect, leftsided diaphragmatic hernia, aplasia of the gallbladder, and typical craniofacial manifestations (bilateral cleft lip and palate, hypertelorism with broad base of nose, low-set ears). Conventional karyotyping revealed a mosaic ring-chromosome 4 with breakpoints tentatively assigned to 4p16 and 4q33. We report on the breakpoint mapping by FISH using probes from subtelomeric regions 4p and 4q, probes for the WHS critical region and for 4q33. An attempt was made to correlate the cytogenetic and molecular data with the histopathological findings. We propose that a thorough work up of human aborted fetuses with WHS is an important strategy to complement data obtained from mouse knock-out models.

**Molecular cytogenetic studies in a family with a half cryptic translocation involving the Wolf-Hirschhorn syndrome critical region.** *S.K. Gogineni<sup>1</sup>, I. Wieting<sup>1</sup>, A. Shanske<sup>2</sup>, R.W. Marion<sup>2</sup>, D. Iacoboni<sup>3</sup>, S.J. Gross<sup>1</sup>, H.M. Nitowsky<sup>1</sup>, S.M. Carter<sup>1</sup>, R. Ehrenpreis<sup>1</sup>, Y. Goldberg<sup>1</sup>, P. Bobby<sup>3</sup>, V.R. Pulijaal<sup>1</sup>.* 1) Genetics Laboratory, Montefiore Medical Ctr, Bronx, NY; 2) Dept of Pediatrics, Montefiore Med Ctr, Bronx, NY; 3) Jacobi Medical Center, Bronx, NY.

The proband is the first born to a 29 yr old mother and 30 yr old father. Maternal complications during the pregnancy includes a positive triple screen and polyhydromnios, and amniocentesis revealed an apparently normal 46,XY chromosome complement. The proband was born at term with moderate hypospadias, mild dysmorphic features and undescended left testicle. High resolution chromosome studies postnatally exhibited an additional band in one of the homologues of chromosome no. 4 in the short arm at the p16.3 band - 46,XY,add(4)(p16.3), in the Wolf-Hirschhorn syndrome (WHS) critical region. Parental chromosome studies revealed that the mother has a similar add(4) chromosome, indicating in that this add(4) may represent a half cryptic balanced translocation. Fluorescence in situ hybridization (FISH) studies using a 4p16.3 locus specific probe for the WHS critical region revealed a deletion of this region in the proband. In the mother there were two signals for 4p16.3 probe, one on chromosome no. 4 and the other is on one of the homologues of chromosome no. 12 at p13.31. These findings were confirmed by whole chromosome painting probes for chromosomes number 4 and 12 which revealed that the proband has a unbalanced karyotype with monosomy for the 4p16.3@ pter region, and trisomy for the 12p13.31@ pter region, i.e. 46,XY,add(4)(p16.3).ish der(4)t(4;12)(p16.3;p13.31)mat(wcp12+,DS496-,D4S174+). The mother has a half cryptic balanced translocation, i.e. 46,XX,add(4)(p16.3).ish der(4)t(4;12)(p16.3;p13.31)(wcp12+,DS496-,DS174+;wcp4+,DS496+). Further molecular studies are in progress seeking genotype-phenotype correlations with the WHS critical region and 12p.

**Analysis of 32 supernumerary marker chromosomes derived from chromosome 15.** *K. Eggermann<sup>1</sup>, U.A. Mau<sup>2</sup>, G. Bujdoso<sup>3</sup>, E. Koltai<sup>3</sup>, T. Eggermann<sup>1</sup>, R. Raff<sup>4</sup>, R. Schubert<sup>4</sup>, G. Schwanzitz<sup>4</sup>.* 1) Institute of Human Genetics, RWTH Aachen, Aachen, Germany; 2) Dept. of Medical Genetics, University of Tuebingen, Germany; 3) Institute of Forensic Medicine, Semmelweis University Budapest, Hungary; 4) Institute of Human Genetics, University of Bonn, Germany.

Among phenotypically normal individuals supernumerary marker chromosomes (SMCs) occur at a frequency of approximately 0.72 per 1,000; in the mentally retarded their frequency may increase to up to 3.27 per 1,000. With an incidence of more than 50 per cent, derivatives of chromosome 15 (der(15)) represent the most common SMCs. The formation mechanism is usually a homologous recombination between two chromosomes 15 leading to a psu dic (15;15) or, more commonly, inv dup(15). We have analysed in detail 32 SMCs derived from chromosome 15; they were either detected pre- or postnatally using conventional cytogenetic and molecular cytogenetic procedures. The following conclusions can be drawn: The vast majority of der(15) were dicentric inv dup(15) with the second centromere being inactive and they were mitotically stable. Presence of euchromatin invariably leads to psychomotor and mental retardation (15q12 syndrome) while exclusively heterochromatic marker chromosomes may be regarded as harmless. However, we experienced a high incidence of heterochromatic der(15) in azoospermic males karyotyped prior to ICSI. In the literature, prevalence of maternal transmission of familial der(15) has been reported. Interestingly, in our cohort paternal inheritance of familial der(15) is more frequent than it would therefore have been expected. To the best of our knowledge, this is the largest number of der(15) examined so far and the results of this study may have implications for genetic counseling.

**Heterochromatic insertion and inactivation of PBGD; a novel etiology for Acute Intermittent Porphyria (AIP).**

*M.A. Fox, R.K. Iyer, Z. Jiang, P.N. Rao.* Univ California Med Ctr, Los Angeles, CA.

Acute intermittent Porphyria (AIP; OMIM 176000) is an AD disorder caused by haploinsufficiency of Porphobilinogen deaminase (PBGD). Very rarely, individuals homozygous for loss of PBGD function (usually compound heterozygotes for defective alleles) have been reported but these are usually fatal early in childhood. Over 130 different mutations have been identified in AIP. Most are single base substitutions resulting in non-conservative amino acid changes, while a smaller number cause splice site defects or premature terminations. A few frameshifting insertions or deletions have also been documented. In this report we describe a now 16-year old male with mental retardation, multiple congenital anomalies, and autism, exhibiting many symptoms suggestive of AIP (constipation, abdominal pain, rust-colored urine, anemia, mild tics and neuropathic changes), with an unusual insertion of heterochromatic material within the chromosomal band 11q23.3 [ins(11;?)(q23.3;?)], the genomic location of PBGD. Molecular cytogenetic analyses showed this material is composed of non-chromosome 11 material (WCP11-), C-band positive but, negative for common alpha-sat. centromeric sequences, and is distal to the MLL gene on 11q23.3. Chromosomal analyses of this proband and other family members revealed that the derivative 11 chromosome is maternally inherited and is also present in the maternal grandfather. However, neither showed any symptoms of AIP. Possible explanations for this include: (1) Heterochromatization of the region, adversely affecting expression of genes in the region, including several potential players in neurodevelopment and/or function; (2) Alteration of the chromatin structure may also have created/alterd local patterns of sex-specific imprinting, and may account for lack of disease in the maternal progenitors; (3) In addition to the 11q23.3 insertion the patient may also contain a deleterious mutation in the paternally inherited PBGD allele; (4) The disease in the patient is the result of a combination of both the effect of the insertion and in utero insults and/or post-natal environmental factors. Mutation analysis of the AIP gene is currently in progress.



**Paternal exposure to cyclophosphamide induces chromosomal damage and differential DNA repair gene expression in mouse zygotes and 2-cell embryos.** *L. Cosentino<sup>1</sup>, F. Marchetti<sup>1</sup>, S. Mabery<sup>1</sup>, J. Bishop<sup>2</sup>, A.J.*

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The integrity of germ cell DNA is critical for the fate of the offspring, yet little is known of the DNA repair capabilities of these cells. Post-meiotic male germ cells lose the ability to repair their DNA while oocytes are equipped with DNA repair mechanisms throughout their maturation. The survival of the zygote strongly depends on maternal mRNAs and proteins stored in the egg before fertilization. The shift from maternal stored gene products to zygotic expression is poorly understood, especially for the genes involved in DNA repair pathways. Also paternally transmitted chromosomal damage may alter the expression of these genes. The purpose of our research was to determine (a) whether paternal exposure to cyclophosphamide (CP) leads to an induction of chromosomal aberrations in mouse zygotes and 2-cell embryos using PAINT/DAPI, and (b) whether fertilization with mutagenized sperm alters the expression profiles of various DNA repair genes during early mouse embryogenesis using real time TaqMan PCR. When B6C3F1 males were mated with untreated females 10 days after CP treatment (120 mg/kg) a significant increase in the incidence of chromosomal aberrations was detected in zygotes ( $36 \pm 4.1\%$ ) and 2-cell embryos ( $49 \pm 1.3\%$ ) compared to controls. Approximately a 2-fold increase in Ku80 and Rad54 transcripts were detected in zygotes fertilized by mutagenized sperm compared to controls, while no significant difference was seen in embryos. These results suggest that CP-induced chromosomal damage is associated with changes in expression of DNA repair genes. These changes appear to be restricted to the first cell cycle after fertilization, when the egg is attempting to repair sperm DNA damage. This work was conducted under the auspices of the US DOE by the University of California, LLNL under contract W-7405-ENG-48 with support from NIEHS ES09117-02 and DOE KP110202.

**A girl with inversion chromosome 7q22-q11.21: Insights into mechanisms of Williams syndrome and**

**ectrodactyly.** *T.A. Grebe<sup>1</sup>, S.R. Cox<sup>1</sup>, J.A. Hebrick<sup>2</sup>, S.W. Scherer<sup>2</sup>.* 1) Ped, Div Medical/Molec Gen, Univ Arizona, Phoenix, AZ; 2) The Hospital for Sick Children, Toronto, Ontario, Canada.

Williams syndrome (WS) and ectrodactyly, while distinct clinical entities, are both linked to chromosome 7q. WS is caused by a deletion of 7q11.23 and is associated with supravalvular aortic stenosis, typical facies, hypercalcemia, and a specific psychobehavioral profile. Ectrodactyly (split hand/split foot) is a defect in formation of the central ray, due to mutations at the SHFM1 locus on 7q21.3-22.1.

We evaluated a girl who exhibits features of both conditions. She was born at term to a G1P0 mother and was noted at birth to have ectrodactyly of the feet. Her development has been delayed and she has mild mental retardation. The diagnosis of Williams syndrome was not made until age 15 years. On examination her height, weight and head circumference are at the 50th percentile. She has thick hair, mild periorbital fullness, a stellate iris pattern, a wide mouth with full lips and a broad neck with a low posterior hairline. She has 5th digit contractures and ectrodactyly of the feet. She is extremely talkative. An echocardiogram revealed only a patent foramen ovale.

Karyotype showed a paracentric inversion of 7q: 46,XX,inv(7)(q22q11.21). FISH analysis using the ELN probe was, however, negative for a deletion at 7q11.23. Further FISH analysis has shown an inversion of WS critical region, including two flanking duplications. Possible mechanisms for the atypical WS phenotype in this patient include a disruption of a gene near the flanking region or other mutations in that area. Alternatively, the inversion of the WS region may have predisposed her to the paracentric inversion causing her complex phenotype. The ectrodactyly is likely due to a disruption of the SHFM1 locus at the translocation breakpoint.

This case provides insight into mechanisms of WS and illustrates the need to consider chromosome analysis as well as FISH analysis in syndromic patients with atypical findings.

**Inverted low copy repeats and a common 8p23 inversion polymorphism.** *N. Matsumoto*<sup>1,2</sup>, *N. Harada*<sup>1,2,3</sup>, *S. Giglio*<sup>4</sup>, *K. Kuroiwa*<sup>5</sup>, *D.H. Ledbetter*<sup>6</sup>, *N. Niikawa*<sup>1,2</sup>. 1) Human Genetics, Nagasaki Univ Sch of Med, Nagasaki, Nagasaki, Japan; 2) CREST, Japan Science and Technology Corporation, Kawaguchi, Japan; 3) Nagasaki Laboratory, Kyushu Medical Science, Nagasaki, Japan; 4) IRCCS Ospedale San Raffaele, Clinical Molecular Biology and Cytogenetics Laboratory, Milano, Italy; 5) Division of Medical Genetics, Kanagawa Children's Medical Center, Yokohama, Japan; 6) Department of Human Genetics, University of Chicago, Chicago, IL, USA.

In order to characterize a novel, submicroscopic, common inversion polymorphism on 8p23, we constructed a 7-Mb BAC/PAC-based physical map covering the entire 6-Mb inversion and its flanking regions. Low copy repeats (LCRs) with an inverted orientation were identified at both inversion breakpoints. Analysis of two patients with inverted duplication of chromosome 8 [inv dup(8)] showed that the breakpoints of their inv dup(8) were consistent with the 8p23 inversion breakpoints. FISH studies in the general Japanese population revealed that 28 of 50 normal individuals were homozygous for the normal chromosome 8, while 17 and 5 were heterozygous and homozygous for the inversion, respectively. Thus, the allele frequency for the 8p23 inversion in the Japanese was estimated to be 27 %. These findings support the previous hypothesis that the 8p23 inversion is causally related to inv dup(8) chromosomal rearrangement.

**An abnormal phenotype displayed in a proband with an inherited “balanced” t(12;16) translocation.** *L. Mehta<sup>1</sup>, A. Babu<sup>2</sup>, S. Kleyman<sup>2</sup>, S. Sastry<sup>1</sup>, M.J. Macera<sup>2</sup>.* 1) Dept. of Pediatrics, Div. of Medical Genetics, North Shore Univ. Hospital, Manhasset, NY; 2) Department of Molecular Medicine and Genetics, Wyckoff Heights Medical Center, Brooklyn, NY.

A female child was referred for genetic evaluation because of a clinical phenotype consisting of relative microcephaly (head circumference 2%), dysmorphisms consisting of frontal prominence, shallow orbits, flat nasal bridge, downturned mouth, high palate, micrognathia, 5th finger clinodactyly and significant 2/3 toe syndactyly. She had a right multicystic kidney and recurrent left knee dislocation with left hip dysplasia. There were mild fine and gross motor delays with no cognitive issues at age 6 years. The child was known to carry a maternal balanced reciprocal translocation 46,XX, t(12;16)(q22;q22.1). The mother was phenotypically normal with both parents having normal head sizes. High resolution GTG banding was performed on chromosomes from both the mother and proband to determine if any material was missing from the child's chromosomes. No apparent loss of material was detected by cytogenetic analysis. The breakpoint on chromosome 16 at q22.1, is a known fragile site. Wenger et al [1995], have reported six cases where abnormal offspring were determined to have inherited apparently "balanced" translocations from normal carrier parents. The breakpoints in these cases were coincident with chromosomal fragile sites. An association has been implied between chromosomal breakpoints and fragile sites, suggesting predisposition of these fragile sites to breakage and unequal cross over. The products of the unequal crossing over could be submicroscopic deletions and duplications. Thus, an apparently "balanced" inherited translocation may be associated with clinical abnormalities. These cases raise a counseling issue in terms of predictive risk/reassurance when dealing with a pregnancy involving a familial balanced translocation. Additional studies are needed to elucidate whether the breakpoints coincident with known fragile sites have a different risk factor when compared to other translocations.

**Interstitial microdeletion of 21q.** *K.M. May, N. Longo.* Emory University School of Medicine, Department of Pediatrics, Division of Medical Genetics, Atlanta, GA.

Partial monosomy for 21q22 has been associated with microcephaly, IUGR, mental retardation, congenital heart disease, hypertonia, short stature and dysmorphic facial features. There is evidence that deletion within the 21q22.2 band may specifically be responsible for microcephaly and IUGR. We present a child who was evaluated at 20 months of age for microcephaly (head circumference (HC) appropriate for 5 month old), short stature (appropriate for 11 month old), developmental delay and febrile seizures. He was the product of a full term pregnancy with a birth weight of 3.75 kg (75th centile). He had coarse facial features with hirsutism, low set and posteriorly rotated ears, thick lips, widely spaced teeth, high arched palate, micrognathia, and hypotonia alternated to hypertonia. Chromosome analysis at 700 bands was interpreted as normal. At repeat evaluation at 4 years he was non-verbal, his height and weight were appropriate for a 2 year old child, and his HC was appropriate for a 10 month old. Subtelomeric FISH analysis with the Vysis, Inc. ToTelVysion probe panel was performed. All subtelomeric probes showed a normal pattern; however, one 21 was missing a signal for the AML1 control probe in 21q22.2. A second hybridization with the Vysis, Inc. LSI21 probe, specific for loci D21S259, D21S341 and D21S342, showed a normal hybridization pattern. These loci are mapped to band 21q22.2, distal to the AML1 locus. Repeat chromosome analysis at 850 bands revealed a subtle change in the size of band q22.2 on one chromosome 21. Data from FISH analysis confirms the distal breakpoint within q22.2. The proximal break could be within q22.13 or in q22.2 on the basis of chromosome analysis. Parental FISH studies were normal. The patient's phenotype supports the theory that a gene or genes responsible for microcephaly reside in 21q22.2. This is, to our knowledge, the smallest reported interstitial deletion of 21q22, and the fact that the child had a normal birth weight followed by postnatal growth retardation may indicate that his proximal breakpoint lies outside of the region responsible for IUGR.

**Screening for cryptic chromosomal abnormalities using telomere FISH probes** . *B.J. Hulley, M. Hummel, S.L. Wenger.* West Virginia University School of Medicine, Morgantown, WV.

Mental retardation is present in 2-3% of the general population. Chromosomal abnormalities may be detected in up to 35% of patients with mental retardation, depending on patient selection and laboratory techniques. Deletions of 2-3 megabases and larger are usually microscopically visible. Smaller deletions, duplications, or rearrangements have been identified in 7% of individuals with idiopathic mental retardation using telomere probes. Some subtle subtelomeric deletions such as 1pter, 1qter, and 22qter are each associated with a specific phenotype including mental retardation. To date 15 blood samples for patients with mental retardation and facial dysmorphism have been collected and analyzed by G-banded karyotypes and telomere FISH. Of these samples, one was sent out for additional molecular testing and was diagnosed with a single gene defect, and two were found to have chromosomal abnormalities: dup(16)(p11.1 p11.2) de novo and del(17)(p11.2 p11.2). All others were apparently normal by karyotype. The three samples were not included in the idiopathic mental retardation group since the cause for mental retardation and dysmorphism was determined. Therefore, out of the remaining twelve cases, two samples were found to have a subtle telomere abnormality: del(1)(p36.3) and der(1)t(1;8)(p36.3; p23). This resulted in 2/12 or 16.7% of idiopathic mental retardation patients having a subtle telomere deletion or rearrangement. Our results suggest that these patients should be routinely evaluated for subtle chromosomal rearrangements using FISH telomere probes. Also, further studies may identify common telomere regions involved in abnormal cases and may lead to clinical phenotype correlation for the characterization of subtle chromosomal syndromes.

**Mosaicism for two de novo supernumerary markers derived from chromosomes 18 and 13.** *K.N. Nandi<sup>1</sup>, M.T. McDonald<sup>2</sup>, K.K. Rogers<sup>1</sup>, K.W. Rao<sup>1</sup>.* 1) Departments of Pediatrics & Pathology, UNC Hospitals; Chapel Hill, NC; 2) Division of Medical Genetics, Duke University Medical Center; Durham, NC.

We present a 4y4m old female with developmental delay and dysmorphic features who showed mosaicism for two different de novo markers originating from chromosomes 18 and 13, respectively. She was born at term (bw: 6lb 1oz) to a 30y G3 mother following an uncomplicated pregnancy; there were no major neonatal problems. She exhibited slight delay in motor development and more marked delay in speech and language development; at age 4yr, she only had a few single words. Her brain MRI, renal US scan, EEG and hearing tests were normal. She appeared to be a healthy child with average height, weight and head circumference. She had a medial flare to the eyebrows, synophrys, long eyelashes, full cheeks, hyperconvex fingernails, bilateral 5th finger clinodactyly, a large space between the first and second toes, a medially deviated second toe, deep plantar creases between the first and second toe, and prominent calcaneus. Chromosome analysis of blood revealed a mosaic karyotype with two supernumerary markers. The markers appeared to be differently sized ring-chromosomes. The larger marker was about half the size of a G-group chromosome, while the smaller marker was about half the size of the larger marker. Of the 50 metaphase cells studied, 27 had one ring, 16 contained two rings, and the remaining 7 cells were normal. C-banding suggested that both markers contained euchromatic and centromeric material. Cytocell's Multiprobe whole chromosome paint FISH assay showed that the large and small marker were derived from chromosomes 18 and 13 respectively. Whole chromosome paints from Oncor confirmed the chromosomal origin of both markers. The larger marker contained both 18p and 18q material as shown by 18p and 18q partial paint FISH probes from American Laboratory Technologies. This patient is therefore mosaic for partial trisomy 18 and partial trisomy 13. We are aware of only one similar case (Plattner et al., 1993). The patient had two supernumerary markers derived from 18 and 13/21 and multiple congenital abnormalities including cardiac, skeletal, and genital-urinary malformations.

**Trisomy for the 16p telomere: genotype/phenotype correlations.** *C.M. Lese, J.F. Hedrick, J.A. Roseberry, A. Wong, S. Uhrig, D.H. Ledbetter, D. Waggoner.* Univ. of Chicago, Dept. of Human Genetics, Chicago, IL.

It has become increasingly evident that a significant proportion of individuals with idiopathic congenital malformations and mental retardation have subtle rearrangements involving the telomeres of human chromosomes. During the course of genome-wide telomere screening in patients with unexplained mental retardation and normal karyotypes, we identified three cases where the telomere rearrangement resulted in trisomy for the 16p telomere. One case involved an unbalanced translocation between the telomeres of chromosome 7p and 16p while the other two unrelated cases had unbalanced translocations involving the 10q and 16p telomeres. In all three cases, only 16p trisomy was observed; the breakpoint on the second chromosome occurred distal to the telomere probe and did not result in any detectable corresponding monosomy. A fourth case, an unbalanced translocation between 16p and 17p, resulting in trisomy 16p and monosomy 17p, was previously reported by our group. Clinical features common to these four patients include: mental and growth retardation, brain malformations, heart defects, cleft palate, pancreatic insufficiency, genitourinary abnormalities and similar dysmorphic features. The clinical diagnoses of Toriello-Carey and Johanson-Blizzard syndromes were considered in two of the cases, demonstrating that the features of trisomy 16p overlap with other conditions. To compare the extent of trisomy and determine genotype/phenotype correlations for these four cases, we developed a molecular ruler for 16p consisting of BAC/PAC clones spaced at ~1 Mb intervals for use in a FISH assay. The size of the trisomic segment ranged from ~4 Mb to ~10 Mb and correlated with the phenotypic severity. Interestingly, the breakpoint of the two 10q;16p translocations was contained within the same BAC clone, suggesting a common mechanism for their origin. The identification and characterization of such cryptic translocations is important for accurate genetic counseling and recurrence risk assessment. In addition, fine mapping to examine genotype/phenotype correlations can help not only to predict clinical outcomes, but also to identify the genes contributing to the phenotype.



**Use of single-sperm typing to determine the relationship between recombination in the pseudoautosomal region and paternal age.** *R.H. Martin*<sup>1,2</sup>, *Q. Shi*<sup>1,2</sup>, *E. Spriggs*<sup>3</sup>, *A.W. Rademaker*<sup>4</sup>. 1) Medical Genetics Clinic, Alberta Children's Hospital, Calgary, Alberta, Canada; 2) Department of Medical Genetics, University of Calgary, AB, Canada; 3) Department of Clinical Chemistry, Health Sciences Centre, Winnipeg, MB, Canada; 4) Cancer Center Biometry Section, Northwestern University, Chicago, IL.

Recombination between the X and Y chromosome is limited to the pseudoautosomal region and is necessary for proper segregation of the sex chromosomes during spermatogenesis. Failure of the sex chromosomes to disjoin properly during meiosis can result in individuals with a 47,XXY constitution and approximately one-half of these result from paternal nondisjunction at meiosis I. Analysis of these paternally-derived 47,XXY cases have shown that the majority of these males are the result of meiosis wherein the X and Y chromosomes have failed to recombine. Our studies in sperm have demonstrated that aneuploid 24,XY sperm have a decreased recombination frequency compared to normal sperm. Some studies have indicated a relationship between increased paternal age with 47,XXY offspring and with the production of XY disomic sperm, whereas others have failed to find such relationships. To determine if there is a relationship between paternal age and recombination in the pseudoautosomal region, single-sperm typing was performed to measure the frequency of recombination between a sex-specific locus, STS/STS pseudogene, and a pseudoautosomal locus, DXYS15, in young men (<30 yr.) compared to older men (>50 yr.). A total of 1599 sperm were typed by single-sperm PCR analysis in 13 men heterozygous for the DXYS15 locus: 708 sperm from 7 young men and 891 sperm from 6 older men. The mean recombination frequency was 38.7% (SE .00865) in the young men and 37.3% (SE .00738) in the older men. The recombination rates were homogeneous in both groups ( $p > .8$ , chi square test). There was no significant difference in the recombination frequencies in young men compared to older men ( $p = .21$ , two-tailed Z test, adjusting for within person clustering of data), suggesting that there is no effect of paternal age on the recombination frequency in the pseudoautosomal region.

**Ring 21 complex mosaicism: clinical and cytogenetic phenotype.** *J. Kreutzman, L. Dong, R.E. Falk.* Med Gen/Birth Defects Ctr, Cedars-Sinai Medical Center, Los Angeles, CA.

MZ was a 2386 gm, 46 cm, 31 cm OFC product of a 38 week gestation to a healthy 30 year old woman and her unrelated 32 year old partner. Prenatal ultrasound suggested IUGR and intracranial cyst. Delivery was by C-section for failure to progress. He had hypoglycemia, apnea with feeding, and GE reflux requiring medical treatment. MRI of the brain revealed a thin corpus callosum and arachnoid cyst. Ophthalmologic evaluation noted foveal hypoplasia OU. Cytogenetic analysis revealed a complex karyotype: 46,XY,r(21)(p10q22[21]/46,XY,del(21)(q21~22)[cp16]/45,XY,-21[6]/46,XY,r(21)(p10q22p10q22[4]. Hence, he had a cell line with ring chromosome 21, cells in which the ring opened at various breakpoints resulting in different 21q deletions, cells with monosomy 21, and cells with a double ring producing trisomy for most of 21q. At age 3 months, his height of 54 cm and weight of 4.3 Kg were 3 SD below the mean, and OFC of 36.5 cm was 4 SD below the mean. He had microcephaly, prominent forehead, bitemporal narrowing and an occipital shelf. Eyes were deep set with downslanted palpebral fissures and long lashes. Ears were asymmetric and mildly dysmorphic. Nose was short with broad bridge, prominent tip and anteverted nares. He had a long philtrum, normal oral exam and retrognathia. The neck, thorax, heart, lungs, abdomen and genitalia were unremarkable. He had lateral elbow dimples, and clinodactyly V. Skin demonstrated cutis marmorata. Neurologic exam revealed moderate, generalized hypertonia, mild developmental delay, and no focal findings or seizures. Developmental assessment at age 12 months revealed cognitive function at 8.5-10 months, language at 5.5-7 months, motor and social skills at 7-8.5 months. Evaluation at 15 months of age revealed length >3 SD below the mean and severe microcephaly. Facial dysmorphism was unchanged except for obvious asymmetry. The hypertonia had improved. He had chronic otitis media requiring middle ear ventilation but no other surgery or hospitalization. Vision and hearing were clinically normal. Detailed cytogenetic characterization and clinical features of this child will be reviewed and compared to those in published cases of r(21).

**Delineation of a BAC contig in chromosome 11p15.5 and detection of a microduplication associated with Beckwith-Wiedemann syndrome.** *L. Jeng, L. Christ, M. Eichenmiller, S. Schwartz.* CWRU and Univ Hosps of Cleveland, Cleveland, OH.

Beckwith-Wiedemann syndrome (BWS) is a congenital disorder, which has been localized to 11p15.5. Patients may present with the classical triad of abdominal wall defect (eg. omphalocele), macroglossia and overgrowth, but in other cases, part of the triad, hypoglycemia and/or Wilms tumor are present. BWS is genetically heterogeneous, but in many cases the etiology is not known. A chromosomal abnormality is estimated to be present in only 2-3% of BWS cases. The most common chromosomal abnormality in BWS is a duplication of the distal short arm of chromosome 11, but only about 20 cases have been described in the literature. Over the past several years, we have used high-resolution chromosome analysis to study 15 referrals with a clinical diagnosis of BWS. Two of these cases were detected to have a de novo chromosome abnormality; one had a duplication of the entire short arm of chromosome 11, and the other had a very subtle duplication of part of 11p15.5. Both cases had features consistent with BWS.

In the second case, cytogenetic analysis allowed only a tentative diagnosis. Therefore, a BAC/STS map was constructed around the putative duplication using 38 BACs and 11 STSs. BACs from this newly constructed map were then used to study the region in question, and the presence of a subtle duplication was confirmed. These studies not only confirmed the duplication, but demonstrated that it was an inverted tandem duplication with an estimated size of only 2-3 Mb. The results of these studies are important in that: (1) we have constructed a BAC contig around the BWS critical region; (2) the duplication we identified in our case involved only a 2-3 Mb region, the smallest reported in BWS; (3) given the identification of such a small duplication, the number of cytogenetic abnormalities in BWS is likely under ascertained; (4) subtle duplications may be present in patients who were previously classified as having a normal karyotype; and (5) the BACs identified in this study can be utilized to delineate similar microduplications in other patients in order to determine if a higher proportion of cases actually have a chromosomal abnormality.

**A male child with a 46,idic(Y)(q11.2)/45X mosaicism and autistic disorder.** *B. Niederer*<sup>1</sup>, *S. Kleyman*<sup>2</sup>, *A. Babu*<sup>2</sup>, *M.J. Macera*<sup>2</sup>. 1) Queens-Long Island Medical Group, P.C., Ronkonkoma, NY; 2) Department of Molecular Medicine and Genetics, Wyckoff Heights Medical Center, Brooklyn, NY.

The etiologies of autism and the related pervasive developmental disorder (PDD) are unknown, however, the contribution of complex genetic factors are strongly implicated. Involvement of chromosomes 4, 7, 10, 16, 19, 22 and the X with these disorders has been reported. [Vincent et al, 2000, Gillberg 1998] There is also growing evidence for an association with autism, PDD and individuals possessing the 47,XYY karyotype. The additional Y may contribute to abnormal brain development, possibly predisposing these children to learning disorders [Nicolson et. al., 1998]. The isodicentric(Y), may be the genetic equivalent to two Y chromosomes. Blackman et al. [1991] did report a case of autism in a child with an 46,X,idic(Y). An 8 1/2 year old boy was referred for genetic evaluation because of developmental delay with autistic features. He was the product of a normal pregnancy, born at term, to a 33 year old mother and 36 year old father. His infancy and early childhood were unremarkable. His motor milestones were normal. He grew at the 5th percentile for height and weight until age 2 years. From that time, his height remained at the 5th percentile, but his weight increased until at age 6, it reached the 95th percentile. His physical examination was within normal limits and he had normal male genitalia with normal testes. By age 5, he exhibited aggressive behavior and poor attention span and was determined to have markedly delayed speech. At age 7, he was placed in a program for autistic children and has done well. Chromosomal evaluation with GTG banding identified a 46,X,Ynf[95]/45,X[5] mosaicism in his peripheral blood lymphocytes. Molecular analysis with probes specific for the SRY gene, Y centromere and Y satellite I DNA (Vysis Inc.) using the FISH technique, revealed a 46,X,idic(Y)(q11.2).ish Y(SRY++, DYZ3++, DYZ1-)/45,X karyotype. The father had a normal Y chromosome [46,XY.ish Y(SRY+, DYZ3+, DYZ1+)]. It is believed that this proband is the first reported case of mosaic 46,X,idic(Y)/45,X with autism. Additional data may elucidate the role of the idic(Y) chromosome in autism.

**Unstable centromeric fission products of a balanced Robertsonian translocation in a markedly discordant twin.**

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Mitotic fission of a dicentric Robertsonian chromosome resulted in an unstable chromosome complement that gave rise to mosaic cells of decreased viability and decreased fetal growth. A woman from southern India conceived twins by intrauterine injection of sperm from a southern Indian donor because her husband is azoospermic. Amniocytes sampled at 16 weeks and again at 18 weeks found Twin A had a normal 46,XX karyotype. Twin B had a 45,XY,dic(13;14)(p11.1;p11.1)[106/128]de novo karyotype in a majority of cells. Centromeric fission of this dicentric chromosome in a progenitor cell and nondisjunction of the unstable derivative chromosomes resulted in cells trisomic for 14q and monosomic for 13q or 14q:mos46,XY,-13,+fis(13)(p11.1),-14,+fis(14)(p11.1)[14/128]/45,XY,-13,-14,+fis(14)(p11.1)[4/128]/45,XY,-13,+fis(13)(p11.1),-14[3/128]/47,XY,-13,+fis(13)(p11.1),-14,+fis(14)(p11.1),+fis(14)(p11.1)[1/128]. Centromeric antibody studies distinguished two active centromeres in 13 dic(13;14) chromosomes and one active centromere in 6. Uniparental disomy for chromosomes 13 or 14 was ruled out in both twins and both had a normal targeted ultrasound. Given the uniqueness of these findings the couple was advised of the uncertain but real risk of abnormality in Twin B, and the risk of selective reduction to Twin A. Considering these risks and options, the couple elected to continue carrying both fetuses. At 32 weeks gestation the mother delivered Twin A who weighed 3 lbs 0 oz and Twin B who weighed 2 lbs 0 oz. No other abnormalities were noted. Term cord blood analysis revealed a 46,XX karyotype in Twin A and a 45,XY,dic(13;14) karyotype in all cells of Twin B. Twin discordance in this study can be explained by the de novo translocation prior to fertilization to produce dic(13;14) in twin B followed by centric fission in an early mitotic progenitor cell and loss of cells with unstable chromosomes. This case illustrates that an unstable chromosome complement may result in discordant fetal growth.

**Case report of a male fetus 46,XY,add(22)(p11.2).ish i(22)(q10)[9]/ish dup(22)(q12q13)[37].** *R. Hassan*<sup>1</sup>, *N. Qin*<sup>2</sup>, *C. Guze*<sup>1,3</sup>, *D. Baker*<sup>3</sup>. 1) Dept of OB/GYN, Genetics Unit, King Drew Medical Center, Los Angeles, CA; 2) Quest Diagnostics at Nichols Institute, San Juan Capistrano, CA; 3) CSU Dominguez Hills, Carson, CA.

A 15-year-old female, G1 P0 Ab0 was referred for amniocentesis counseling because of an abnormal ultrasound at 18 weeks gestation. The ultrasound showed an echogenic bowel, irregular heart beat and a left club foot. The family history revealed that the mother of the fetus also had a left club foot that had been repaired. TORCH titers were negative. CF mutation testing on the mother gave her a carrier risk of 1/198. A fetal echocardiogram done at 21 weeks was normal, no fetal arrhythmia was noted. Amniocentesis was performed at 21 3/7 weeks. The AF-AFP was 0.33 MoM. Cytogenetic analysis found additional genetic material of unknown origin attached to the short arm of one chromosome 22. FISH study using whole chromosome 22 painting probe identified the extra material as chromosome 22 in origin. Subsequent FISH studies using the TUPLE1 probe mapping to the DiGeorge syndrome region (22q11.2) with the ARSA control probe mapping to 22q13 revealed an abnormal mosaic karyotype with two cell lines. Among 46 metaphases examined, 9 (20%) had an isochromosome for the long arm of chromosome 22: i(22)(q10)[9]; the remaining 37 metaphases (80%) showed a duplication of a segment distal to the q11.2 region [dup(22)(q12q13)]. Therefore, the resulting net imbalance is trisomy for the long arm of chromosome 22 (20%) and trisomy for the segment distal to 22q11.2 (80%). Chromosome analysis of the mother of the fetus showed a normal 46,XX karyotype. The father of the fetus is phenotypically normal. However, no chromosome study on him is available at this time. The mother chose to continue the pregnancy. Repeat ultrasound at 25 4/7 weeks revealed polyhydramnios. Follow up ultrasounds are scheduled.

**Report of 6 cases of trisomy 10p resulting from parental pericentric inversion (10).** *R.R. Karimi-Nejad, H. Karimi, H. Miri, N. Nabavi-nia, M. Rahimi, K. Shirneshan, M.H. Karimi-Nejad.* Karimi Nejad Path & Genetics Center 1143 Shahrak Gharb Medical Bldg Shahrak Gharb, Tehran 14667 Iran.

During the past 20 years and among more than 25000 peripheral blood karyotypes, we have detected 6 cases of add(10)(q25). All of these cases were referred between 1-12 months of age with multiple congenital anomalies including: severe growth retardation, congenital dislocation of the hip, bilateral club feet, high bossing forehead, narrow face with sagging cheeks, broad nasal bridge, harelip or cleft palate, small round chin, large posteriorly rotated low set ears, hyperflexed limbs, hypoplastic genitalia and severe hypotonia. One of the families did not participate in the study. In 5 of the cases, their parents' karyotypes were studied and 3 of the fathers and 2 of the mothers had a pericentric inversion in chromosome 10. The inversion involves the short and the long arm with breakpoints at p11 and q25, resulting in a long acrocentric chromosome 10. This identified the karyotype of the children as rec(10)dup(10p)inv(10)(p11q25). Prenatal diagnosis has been performed for two of the families, revealing a normal fetus in one and a trisomy 10p in the other.

**Cytogenetic and molecular characterization in a Turner patient with a complex Y chromosome mosaicism.** S.

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The phenotypic spectrum of mosaic individuals with 45,X/46,XY or 46,X,der(Y)karyotype varies from females with Turner syndrome through intersex conditions to almost normal males. Clinical expression, is believed to be dependent on the level of mosaicism in gonadal tissues but this correlation has not been confirmed in all reported cases. Recently, a missense mutation in the 5 non HMG box region of the SRY gene has been described in two patients with Turner syndrome and Y or der(Y) mosaicism (Canto et al., 2000). It has been proposed that such kind of mutations, associated with the predominance of the 45,X cell line in the embrional gonad, is able to prevent the development of any testicular tissue in at least a number of Turner syndrome patients with 45,X/46,XY or der(Y) mosaicism. We describe a patient with Turner syndrome and a complex sex chromosomes mosaic karyotype, in which we performed cytogenetics and molecular studies of the SRY gene. The girl was referred for genetic evaluation at age 9 years because of her short stature. Her height was cm 114 (under 3rd percentile) while her weight Kg 23 (3rd-10th percentile). Hypertrichosis and areas of hyperpigmentation were present on her back and limbs; her external genitalia were female. Cardiac auscultation showed a 2/6 midsystolic murmur. Pelvic ultrasound demonstrated a hypoplastic uterus and a right gonad recognizable with difficulty, while the left gonad was not evident. Cytogenetics analysis on peripheral blood lymphocytes showed the presence of a mosaic karyotype with 4 different cell lines: 45,X[42]/ 46,X,der(Yq)[32]/46,X,idic(Yp)(q11.23)[24]/47,X,idic(Yp)(q11.23),idic(Yp)(q11.23)[2]. FISH analysis was carried out using DYZ3 Oncor probe, 884b7, 913b1, 908e8 YAC probes and PHU (SRY) BAC probe. Sequencing of the whole coding region of SRY gene didn't reveal any mutation suggesting that SRY gene mutations are not always involved in determining of female phenotype in individuals carrying Y chromosome mosaicism.



**Prenatal diagnosis of a small supernumerary, mosaic XIST-negative, ring X chromosome identified by fluorescence in situ hybridization in an abnormal male fetus.** C. LE CAIGNEC<sup>1</sup>, M. BOCENO<sup>1</sup>, M. JOUBERT<sup>2</sup>, N. WINER<sup>3</sup>, F. AUBRON<sup>4</sup>, J.M. RIVAL<sup>1</sup>. 1) SERVICE DE GENETIQUE, CHU, NANTES, FRANCE; 2) LABORATOIRE D'ANATOMIE PATHOLOGIQUE, CHU, NANTES, FRANCE; 3) SERVICE DE GYNECO-OBSTETRIQUE, CHU, NANTES, FRANCE; 4) CENTRE D'ECHOGRAPHIE DE L'ILE GLORINETTE, NANTES, FRANCE.

Abnormal chromosomes whose origin cannot be unequivocally established are called marker chromosomes. Marker or ring X [r(X)] chromosomes of varying size have been found in 5% of Turner syndrome patients. The phenotype of these patients was variable : some had the typical turner syndrome phenotype, while others had a more severe phenotype including mental retardation, and other abnormalities. The more severe phenotype was usually associated with very small r(X) chromosomes which did not include the X-inactivation (XIST) locus. Presumably, the abnormal phenotype was due to functional disomy for the duplicated X chromosome regions. Small r(X) chromosomes are rare in males and there are only five previous reports of such cases. We report the identification of a small supernumerary chromosome in an abnormal male fetus. A 28-year-old woman, gravida 1 presented for first-trimester chorionic villus sampling because of fetal nuchal translucency thickness (4 mm) at 12 weeks gestation. RTG-banding study showed a karyotype 46,XY[4]/47,XY,+r(X)[10]/48,XY,+r(X),+r(X)[6]. FISH with a probe specific for the X centromere (DXZ1 probe) showed the marker to be of X chromosome origin. Subsequent FISH analysis with an XIST locus probe (Oncor) was negative, indicating that the XIST locus was not present in the ring X chromosome. Parental karyotypes from lymphocyte cultures were normal. A second ultrasound examination was performed at 20 weeks gestation and revealed a Dandy-Walker malformation, an agenesis of corpus callosum and septum pellucidum and a hypoplasia of vermis. After genetic counselling, the family decided to have the pregnancy terminated. Autopsy confirmed brain malformations. To our knowledge, we report the first prenatal diagnosis of a small supernumerary, mosaic XIST-negative, ring X chromosome in an abnormal male fetus.

**A targeted ablation of the Mlh3 locus.** *S.M. LIPKIN<sup>1</sup>, V. WANG<sup>2</sup>, D. SHANMUGARAJAH<sup>2</sup>, J. CHENG<sup>2</sup>, E. PAM<sup>2</sup>, J. THOMAS<sup>2</sup>, E. GREEN<sup>2</sup>, J. TOUCHMAN<sup>2</sup>, F. COLLINS<sup>2</sup>, P. COHEN<sup>3</sup>.* 1) Dept of Medicine, UC IRVINE, IRVINE, CA; 2) NHGRI/NIH, BETHESDA, MD; 3) ALBERT EINSTEIN COLLEGE OF MEDICINE, BRONX, NY.

DNA mismatch repair is of considerable scientific and medical importance because of its essential role in maintaining genomic integrity during meiosis and mitosis, and its association with Hereditary Non-Polyposis Colon Cancer. Our laboratory recently cloned MLH3, the 9th mammalian DNA mismatch repair gene, and demonstrated the existence of somatic mutations in MSI+ colon cancers. In order to understand the biological roles of MLH3, we have sequenced the Mlh3 locus from the C57BL/6 mouse strain, and compared and annotated the genomic organization of this sequence with reference to the finished MLH3 locus sequence. We have used this sequence to create a targeted ablation of Mlh3 in the mouse. Provocatively, both Mlh3 male and female deficient mice are infertile, and demonstrate severe defects in meiosis prophase I, including aneuploidy and a meiotic arrest phenotype in male germ cells that is distinguishable histologically from that observed in other DNA mismatch repair gene targeted ablation studies. These results suggest Mlh3 plays a unique role in mammalian meiosis that cannot be compensated for by other MutL homologues.

**Three Way Translocation confirmed by M-FISH in a Couple with Reproductive Loss.** *N.B. Kardon, T.M. Dunn, K. Hirschhorn, B. Levy.* Human Genetics and Pediatrics, Mount Sinai School of Medicine, New York, NY.

A 28yr old G 0020 presented with a history of two early spontaneous abortions. Her husband was 32 years old. They were referred for chromosome analysis by their obstetrician as part of a routine workup for reproductive loss. Family history was unremarkable. There were no other instances of reproductive loss. Medical history was unremarkable except for a recent diagnosis of cavernous angiomas in the husband. Routine chromosome analysis was performed and revealed a normal 46,XX karyotype in the patient. Her husband presented with a three way translocation described as 46,XY,t(1;4;7)(p35;q32.1;p14). The initial analysis was obtained using G-banded chromosomes and molecular cytogenetic techniques were utilized to confirm the origins of the chromosomes involved in the 3-way translocation. Whole chromosome paints and M-FISH revealed that material from the long arms of chromosome 4 had moved to the short arms of chromosome 1; material from the short arms of chromosome 7 had translocated to the long arms of chromosome 4; and material from the short arms of chromosome 1 was located on the short arms of chromosome 7. During gametogenesis, there are 20 possible combinations of these three chromosomes when considering 3:1 segregation and only 2 of these would be expected to be viable. The first viable combination would comprise chromosomes that are not involved in the translocation and the second would contain the same balanced rearrangement as the father. The predicted risk for an unbalanced offspring would be 90%. However, when the adjacent-1 and adjacent-2 meiotic errors are included, the possibility of having an unbalanced offspring is theoretically closer to 99%. It is common practice to refer patients with more than two spontaneous abortions for chromosome analysis. The yield of finding a chromosome translocation is low by routine cytogenetic techniques, however this case demonstrates the importance of pursuing this line of investigation. With the availability of molecular cytogenetic techniques, it is now possible to identify even more subtle rearrangements that could have the same adverse implications for reproduction.

**Learning difficulties, minor dysmorphic features and interstitial telomeric sequences in a boy with 45,XY and a 5;22 translocation.** A. Krueger, R. Reichl, P. Stankiewicz, I. Hansmann. Institut fuer Humangenetik und Medizinische Biologie, Halle/Saale, Germany.

We report on a 10-year-old boy who was referred for cytogenetic analysis because of moderate learning difficulties, speech problems and attention deficits. As the only dysmorphic features a bulbous nose, low set ears, short philtrum and a short stature (10th percentile) was present. Chromosome analysis of cultured lymphocytes revealed a karyotype of 45,XY,-22 with additional chromosome material on the long arm of chromosome 5 in all metaphases. The karyotype of both parents was normal with 46,XX and 46,XY, respectively. Microscopically there was no evidence for loss of chromosome 5q bands. A chromosome 5 paint labeled the normal 5 and the der(5) with the exception of a distal segment at 5q. A chromosome 22 paint labeled this distal der(5) segment in addition to the normal chromosome 22, indicating a translocation between chromosome 5q and 22q. For narrowing down the translocation breakpoint FISH using probes for the DGS1 locus at 22q11.2 (D22S75) and the control locus (D22S39) was performed. Analysis revealed no signal for the DGSCR at 22q11.2 but signal for the control locus D22S39 at 22q13.3, indicating deletion of the DGSCR. FISH using the all human telomere probe (Oncor) surprisingly identified two sites on the long arm of der(5) chromosome: one at the distal end representing the telomere of chromosome 22q and one interstitial site at a position compatible with the original telomere of the regular chromosome 5q. It is concluded that the mild phenotype associated with the translocation is due to hemizyosity of the DGSCR at 22q11.2. Interstitial telomeric repeats are only rarely observed in the human karyotype. Occasionally they are detected in neoplasia and there in association with jumping translocations. We do not have evidence for instability of the translocation chromosome in cells of our propositus. This could mean that jumping translocations or translocation instability might not be directly related to the existence of interstitial telomeric sites. (P.S. has been supported by DAAD).

**Risk Assessment of Prenatal Double Supernumerary Ring(9) Chromosomes.** *P.N. Mowrey<sup>1</sup>, B. Williford<sup>1</sup>, S. Kratzer<sup>1</sup>, J.H. Tepperberg<sup>1</sup>, I.K. Gadi<sup>1</sup>, K.K. Phillips<sup>1</sup>, J. Canterino<sup>2</sup>, R. Neal<sup>2</sup>, T. Dalaya<sup>2</sup>, P.R. Papenhausen<sup>1</sup>.* 1) Diagnostic Genetics, Laboratory Corp of America, Res Triangle Pk, NC; 2) Jersey Shore Perinatal Institute, Neptune, NJ.

Supernumerary marker chromosomes range in size from tiny dot-like structures to large inverted duplications to complete telomere-telomere rings. Occurrence is ~1/1000 prenatal studies (Hook and Cross, 1987), of which 40% are familial with a typically normal outcome. Approximately 13% of the de novo cases present with an abnormal phenotype (Warburton, 1991). We report a 34 y.o. female referred for AMA, with an obstetric history significant for a previous 35 wk cesarean delivery for oligohydramnios and breech presentation. Ultrasound findings at 17 wks gestation identified a complete placenta previa, but no fetal anomalies were noted. Heart, face and genitalia were not well visualized. Prenatal chromosome analysis revealed the presence of two small supernumerary marker chromosomes in all colonies. Both markers were shown to be completely C-band positive and derived from the chromosome 9. FISH was 1/3 positive for the pericentromeric and 2/3 positive for the heterochromatic regions. There was no hybridization with WCP9, implying absence of euchromatin. The karyotype was 46,XY,ish r(9)x2(D9Z3+, D9Z5+, WCP9-). Maternal chromosome analysis identified a single marker chromosome in 65% of the metaphases analyzed that appeared similar to the small marker chromosome observed in the fetus. UPD studies confirmed biparental inheritance of the normal chromosome 9 homologues. Markers of this type are usually considered not to be clinically significant when inherited from a clinically normal parental carrier. However, this conclusion must be tempered by both the possibility of increased dosage effects from two markers and the non-mosaicism in the fetus. Although this fetus carried about 3X the maternal extra dosage of this material, the risk of congenital anomalies would appear to be low based on the apparent heterochromatic derivation of the marker chromosome. Sparse case reports also support an apparent tendency for chromosome 9 and 16 derived rings to be composed of benign alpha and classical satellite DNA.

**Common chromosomal anomalies detected in mouse embryonic stem cell (ES) cell lines.** *S. Li, F. Bates, J.J. Mulvihill.* Dept Pediatrics, Univ Oklahoma Hlth Sci Ctr, Oklahoma City, OK.

The ability to obtain a germ line is one of the most important steps in the process of developing a transgenic or knockout mouse with the targeted mutated gene of interest. A common problem with this technology is that the ES cells often lack or have a low efficiency of germ line transmission. To find out whether chromosomal anomalies are correlated with a low efficiency of ES cell germ line transmission, we examined 56 ES cell lines with different constructs at different cell passages using conventional cytogenetics analysis as well as fluorescence in situ hybridization (FISH). Chromosomal abnormalities occurred in 31(55%)specimens: 20 had trisomy 8 or mosaic trisomy 8, 8 had partial trisomy 8 resulting from unbalanced translocations, and 3 had other chromosomal anomalies (trisomy 12, translocation between chromosomes 6 and 12, and random loss or gain of chromosomes). ES cell lines with full or partial trisomy 8 can contribute to somatic tissues, but lose the ability to contribute to the germ line. Our data indicate that karyotyping analysis is a very important tool in quality assurance and perhaps improving the yield of gene targeting experiments in mice. Further, the cytogenetics abnormalities may shed light on the mechanisms of fetal wastage and aneuploidy.

**Partial monosomy 4p with no overt phenotype of Wolf-Hirschhorn syndrome and/or Pitt-Rogers-Danks syndrome.** *L.H. Lockhart<sup>1</sup>, J. Hawkins<sup>1</sup>, S. Zhang<sup>1</sup>, V.S. Tonk<sup>2</sup>, G.V.N. Velagaleti<sup>1</sup>.* 1) Dept of Pediatrics, Univ. Texas Medical Branch, Galveston, TX; 2) Dept of Pediatrics, Texas Tech Univ., Lubbock, TX.

Wolf-Hirschhorn syndrome (WHS) and Pitt-Rogers-Danks syndrome (PRDS) are multiple malformation syndromes resulting from a partial deletion of p16 region of chromosome 4 [del(4)(p16)]. There is wide variability in both the range and severity of symptoms. However, the extent and severity of phenotype are correlated with the length of the deleted segment. Here, we report two cases of partial monosomy 4p detected prenatally. In both cases the phenotypic features were not typical of those seen in the WHS or PRDS.

Case 1 involved a 21-year-old, Caucasian woman who underwent amniocentesis because of multiple fetal anomalies observed on ultrasound at 17 weeks gestation. The fetus had cleft lip and palate, increased nuchal thickness, cystic hygroma, cervical hemivertebrae, a small meningomyelocele, left pleural effusion, diaphragmatic hernia, two-vessel cord, ventricular septal defect and clubbed feet; features commonly seen with trisomy 13. Chromosome analysis showed extra material on the terminal short arm of a chromosome 4 [46,XX,add(4)(p16)]. FISH studies with WHS probe showed that the tested locus was deleted. Parental chromosome analysis showed father with a balanced karyotype 46,XY,t(4;13)(q32;p16), which was confirmed by whole chromosome painting. Thus the fetus has partial monosomy 4p16->pter and partial trisomy 13q32->qter [46,XX,der(4)t(4;13)(p16;q32).ish der(4)del(4)(p16.3p16.3)(WHS-).

Case 2 involved a 35-year-old G7P3A3, Hispanic woman who underwent amniocentesis since she was known to carry a balanced translocation with karyotype 46,XX,t(4;12)(p15.3;p13). She had one child with phenotypic features similar to WHS. Chromosome analysis from amniotic fluid showed partial monosomy 4p [46,XX,der(4)t(4;12)(p15.3;p13)]. However, the fetus did not show any abnormalities on ultrasound at 20 weeks gestation except for increased nuchal thickness.

Both couples elected to terminate the pregnancies and refused autopsy or examination of the fetus.

**Chromosome 15 duplicons mediate both Class I and Class II common deletions of Prader-Willi and Angelman syndrome patients.** *S.K. Mewborn<sup>1</sup>, J.A. Fantes<sup>2</sup>, N.L. Miley<sup>1</sup>, R. Brown<sup>1</sup>, S.L. Christian<sup>3</sup>, D.H. Ledbetter<sup>1</sup>.* 1) Dept of Human Genetics, Univ of Chicago, Chicago, IL; 2) Medical Genetics Section, Department of Medical Sciences, University of Edinburgh, Edinburgh, UK; 3) Department of Psychiatry, University of Chicago, Chicago, IL.

Molecular analyses of Prader-Willi and Angelman syndrome patients provided evidence that the breakpoints of the deletions clustered to three locations; one distal location (BP3), and two proximal (BP1 and BP2). These breakpoints create two classes of patients, Class I using BP1 and Class II using BP2. Mapping of these breakpoint regions revealed blocks of duplicated genomic sequences or duplicons of approximately 500 kb at BP2 and BP3 in inverted orientation. Breakpoint 1 was hypothesized to contain duplicon sequences as well. Current genomic sequence and mapping information has enabled the construction *in silico* of a contig from BP2 to BP1, spanning approximately 200 kb. Anchor BAC clones containing unique and duplicon sequences confirm the presence of duplicon sequences at BP1 and connect BP1 to the pericentromeric pseudogene amplicon previously mapped to proximal 15q. Analysis of the sequence of these anchor BACs has provided evidence that BP1 is in the same orientation as BP2, but contains only a partial copy of the duplicon found at BP2 and BP3. In order to confirm the involvement of the duplicon sequences in the deletion events, pulsed field gel electrophoresis (PFGE) analysis has been performed to identify junction fragments in Prader-Willi and Angelman syndrome patients. Seven junction fragments have been identified. These junction fragments are not identical in size, but show some clustering suggesting that within the duplicons there may be hotspots of breakage. *in silico*.



**Mapping of a mosaic deletion of the short arm of chromosome 7 (7p15.3 to 7p21.1).** *J. Mack, M. Nimmakayalu, B. Pober, M.B. Qumsiyeh.* Genetics, Cytogenetics, Yale University Sch Med, New Haven, CT.

A deletion involving the short arm of chromosome 7 has been reported to result in variable phenotypes which may include delayed growth and development, cleft palate and dysmorphic facial features such as epicanthal folds, hypertelorism, high forehead and micrognathia. Some patients also had congenital heart disease and craniosynostosis. We investigated a 6 year old female referred for evaluation of her developmental delay and congenital anomalies. Clinical evaluations revealed pulmonary valvular stenosis, cleft palate, bilateral epicanthal folds, wide palpebral fissures with apparent telecanthus, prominent nose, and smooth philtrum. Cytogenetic analysis revealed seven cells with a 46,XX karyotype and 14 cells containing a possible paracentric inversion of 7p15.3p21.3 *de novo*. YAC hybridization instead showed a deletion in 7p15 to 7p21.1 rather than an inversion. YAC 927-h-6 (1140 kb) was deleted and a more proximal YAC (850-a-1, 830 kb) had reduced signal on the deleted chromosome. High resolution GTW-banding showed that the banding pattern of the short arm in this case could be explained by a deletion rather than an inversion. Previous reports of deletions of 7p suggest that there are two bands associating with craniosynostosis on 7p: 7p13 and 7p21. Our case with a deletion of 7p15.3 to 7p21.1 and no craniosynostosis suggest that this phenotype on 7p21 is likely associated with more distal deletions at 7p21.2 or 7p21.3. A deletion of TWIST gene at 7p21 was reported in association with Saethre-Chotzen syndrome (SCS, acrocephalosyndactyly type III). This patient had little similarity to SCS. The presence of several homeobox and other developmental genes in the deleted area of 7p15.3 likely accounts for the phenotypic features seen in our patient.

**Molecular characterization of microscopically visible deletion of 7q11.23 to q21.11.** *M.A. Nimmakayalu, J. Mack, M.B. Qumsiyeh.* Dept Genetics, Yale Univ, New Haven, CT.

Visible deletions of 7q11.2 region are very rare and the two cases reported earlier were not characterized molecularly. Our patient had heart murmur and non-obstructive hypertrophic cardiomyopathy at birth. Motor delay was noticed around 6 months. He was notable for growth delay, mental retardation, seizures, and weak vocalizations but with absent speech. At clinical examination, the proband had microcephaly, prominent lips, microstomia, macrocytosis of the red cells, ataxic gait, and mild supra-aortic stenosis. Karyotype at 550-600 band level showed a microscopically visible deletion: 46,XY,del(7)(q11.23q21.11)de novo. FISH using a BAC probe that includes Elastin and LIMK1 (Vysis Inc.) confirmed a deletion. To further characterize the breakpoints in this visible deletion, YACs were picked from two contigs in the area of the deletion. Of the seven YACs we mapped in this patient's sample, one (771A1) was deleted, three mapped proximal, and three mapped distal to the deletion. Our molecular characterization suggests a large deletion of more than 3 Mb. The patient's phenotype represents an extension of the Williams syndrome phenotype. The involvement of mental retardation, absence of speech, seizures, and red blood cell abnormalities are likely due to hemizygosity for genes outside the classical Williams syndrome region. Erythropoietin loss (7q21) may be critical in the red cell macrocytosis.

**Analysis of Genetic Sex Reversal by Comparative Genomic Hybridization.** *B. Levy*<sup>1</sup>, *M. Huang*<sup>1</sup>, *K. McElreavey*<sup>2</sup>, *H. Ostrer*<sup>3</sup>. 1) Mount Sinai School of Medicine, New York, NY; 2) Institut Pasteur, Paris, France; 3) New York University School of Medicine, New York, NY.

Genetic sex reversal occurs with a frequency of 1 in 20,000 individuals. The associated phenotypes are either 46,XY pure or mixed gonadal dysgenesis, 46,XX maleness or 46,XX true hermaphroditism. In approximately 2/3 of cases, the genetic basis is unknown. We hypothesized that some of these cases may arise from chromosomal imbalances (gains or losses) that could be detected by comparative genomic hybridization (CGH). First, we calibrated the technique by analyzing cases of sex reversal with known cytogenetic abnormalities and confirmed 9p and 11p deletions in two cases of 46,XY gonadal dysgenesis. Among several cases with 46,XY gonadal dysgenesis, we observed duplications of the distal short arm of chromosome 9, not seen by conventional cytogenetics, including one case previously reported as a 9p deletion. In a case of 46,XX campomelic dysplasia, we observed an inverted duplication of distal 17p in the region of the SOX20 gene, not seen by conventional cytogenetics. This was confirmed by fluorescence in situ hybridization. In two cases of 46,XX true hermaphroditism, we observed the presence of a Y-bearing cell line, confirming our previous observations that such cases may arise from mosaicism or chimerism. These findings suggest that chromosomal imbalances are an important cause of sex reversal affecting dosage for sex-determining genes. The range of such dosage-sensitive genes extends beyond DAX1, SOX9, and WNT4 and may include genes in the DMRT family. Thus, CGH offers an important new tool for mapping sex-determining genes.

**Unusual X inactivation: an active ring(X).** *M. Shago*<sup>1</sup>, *D. Antinucci*<sup>1</sup>, *P. Chakraborty*<sup>2</sup>, *M. Sgro*<sup>3</sup>, *T. Barozzino*<sup>3</sup>, *D. Chitayat*<sup>2</sup>, *I. Teshima*<sup>1</sup>. 1) Cytogenetics Laboratory, Department of Pediatric Laboratory Medicine, Hospital for Sick Children; 2) Clinical Genetics, Hospital for Sick Children, 555 University Avenue; 3) Division of Pediatrics, Mount Sinai Hospital, 600 University Avenue, Toronto, Canada.

A newborn female with birth parameters (length, weight, and head circumference) of less than the third percentile presented with facial dysmorphism including flattened mid face, a prominent forehead, a small chin, and low set posteriorly rotated ears. Physical assessment revealed short limbs and small hands and feet. Mild pulmonary stenosis was detected and an eye examination indicated Rieger's anomaly. Cytogenetic analysis of cultured lymphocytes revealed a karyotype of 47,X,r(X)(p?q?),der(Y)rea(X;Y)[49]/46,X,der(Y)rea(X;Y)[3] by GTG banding, FISH, C-banding and spectral karyotyping. The mitotic instability of the ring chromosome was evident by its absence in 3/52 cells and the presence of a double ring in 2/52 metaphases examined. The derivative Y appears to be a complex rearrangement of chromosomes X and Y. By FISH, the *SRY* gene determining region is not present on the derivative Y while the *XIST* gene (within the X inactivation locus) is present on both the normal X and the r(X) but not on the der(Y). Replication studies suggest that the normal X is late replicating (inactive) and the r(X) is early replicating (active) in 50/50 cells examined. In normal females, X inactivation is a random event occurring early in embryogenesis. Abnormal Xs lacking *XIST* cannot undergo inactivation. Most abnormal X chromosomes that contain *XIST*, although they are randomly inactivated, are late replicating and inactive as a result of secondary cell selection. The unusual skew of activation towards the r(X) in this case presumably results in the least amount of functional disomy of X-linked genes in the cells of this patient. By FISH, this child has an Xp terminal deletion. Mutations in the *SHOX* gene, which localizes to the pseudoautosomal region of Xp, lead to short stature and mesomelic limb shortening. FISH analysis using probes from the *SHOX* genomic region revealed that this child has haploinsufficiency of the *SHOX* gene.

**Deletion of chromosome 15 centromere and short arm sequences in a patient with features of Prader-Willi syndrome (PWS).** *C.M. Tuck-Muller<sup>1</sup>, J.E. Martinez<sup>1</sup>, G. Lu<sup>2</sup>, E.R. Rowley<sup>1</sup>, A.K. Lee<sup>1</sup>, W. Wertelecki<sup>1</sup>, T.J. Chen<sup>1</sup>.* 1) Dept Medical Genetics, Univ South Alabama, Mobile, AL 36688; 2) Dept Obstetrics and Gynecology, Univ South Carolina, Columbia, SC 29203.

About 70% of PWS patients have deletions in chromosome 15q which include the SNRPN gene. We report a female with some features of PWS and deletion of chromosome 15 centromere and proximal short arm sequences. Examination at age 9 years revealed tall stature, obesity, hypotonia, distally tapered fingers, almond shaped eyes, narrow bifrontal diameter, micrognathia, large ears with fleshy lobes and mental retardation. She had a normal DNA methylation pattern at the SNRPN locus, suggesting that she is not a typical PWS patient. The patient's mother, who was unavailable for examination, is also obese and is said to be "slow". No abnormalities were detected in either the patient or her mother by high resolution chromosome analysis. The FISH probes SNRPN, specific for the PWS region (15q11-q13), and PML (15q22) hybridized to both copies of chromosome 15, while the probes D15Z4, specific for the centromere, and D15Z1, specific for the proximal short arm (15p11.2), failed to hybridize to one copy of chromosome 15 in both the patient and her mother. To rule out a translocation between this chromosome 15 and a non-homologous chromosome as the reason for the failure of hybridization, we used individual centromeric probes specific for all chromosome pairs (Cytocell Chromoprobe Multiprobe system). Two hybridization signals were seen with each probe, except for the chromosome 15 probe where only one signal was seen and the probes for chromosomes 13 and 21 and chromosomes 14 and 22 where cross-hybridization produced 4 signals. These results provide no evidence of a translocation. We conclude that the failure of hybridization with centromeric and short probes is probably due to deletion of repetitive DNA sequences in the unusual chromosome 15, which may represent a normal chromosome variant. However, if the deletion extends into the proximal long arm, it may be associated with the abnormal phenotype in the patient and her mother.

**Phenotypic effects of tetrasomy 20p.** *M. Thangavelu*<sup>1</sup>, *I.N. Zeibarth*<sup>2</sup>, *S. Bhatt*<sup>1</sup>. 1) Genzyme Genetics, Orange, CA; 2) Kapiolani Medical Center for Women and Children, Honolulu, HI.

Structurally abnormal chromosome 20, resulting in partial trisomy 20 has been observed both as derivatives and as markers. We present a case of mosaic tetrasomy 20p diagnosed prenatally. Amniocentesis and cytogenetic studies were performed on a 43 year old because of advanced maternal age and abnormal maternal serum screen indicating an increased risk for trisomy 21. An extra marker chromosome of de novo origin, identified as an idic(20)(q11.2) (isodicentric for the short arm of chromosome 20) by conventional cytogenetics and FISH, was observed in 11 of 15 colonies. At 33 weeks, because of decreased fetal movement and heart deceleration the baby was delivered by emergency C-section. During a three month hospitalization, the patient was found to have bilateral inguinal hernia, bilateral cryptorchidism, left pelvic kidney, gastrointestinal reflux and mild dysmorphic features (rotated ears, long philtrum, micrognathia, and prominent occiput). To our knowledge this is the first case of tetrasomy for 20p. As in previously reported cases of abnormalities resulting in mosaicism for increased dosage of chromosome 20 material as the sole abnormality, the phenotypic abnormalities appear to be minimal. Although there is anecdotal evidence to suggest a probable relationship between chromosome 20 aneuploidy and abnormalities involving the kidney and gastrointestinal system, additional studies are required at the epidemiological as well as biological level.

**Screening cryptic telomeric rearrangements in children with idiopathic mental retardation using an automated fluorescent genotyping strategy.** *M. RIO<sup>1</sup>, S. HEUERTZ<sup>1</sup>, F. MOLINARI<sup>1</sup>, V. PIGNOT<sup>1</sup>, C. TURLEAU<sup>1</sup>, MC. de BLOIS<sup>2</sup>, O. RAOULT<sup>2</sup>, M. PRIEUR<sup>2</sup>, S. ROMANA<sup>2</sup>, A. VEKEMANS<sup>2</sup>, A. MUNNICH<sup>1</sup>, L. COLLEAUX<sup>1</sup>.* 1) INSERM U393, Hopital NECKER-ENFANTS MALADES, PARIS, FRANCE; 2) Service de Cytogenetique, Hopital NECKER-ENFANTS MALADES, PARIS, FRANCE.

Mental retardation is a common condition that affects largely 2 % of the general population. However, its origin remains poorly understood. Recent studies have demonstrated that cryptic unbalanced subtelomeric rearrangements contribute to a significant proportion of idiopathic mental retardation cases. Because of the limited sensitivity of routine analyses, we developed a novel strategy based upon automated fluorescent genotyping to search for non-Mendelian segregation of telomeric markers. The results of a pilot study suggested that fluorescent genotyping is a sensitive and cost-effective method to detect telomeric rearrangements and that the microsatellite technique provides the unique opportunity to detect uniparental disomies. Here we report a larger study including 110 children. This study resulted in the characterization of twelve telomeric rearrangements and two uniparental disomies, confirming the efficiency of this strategy to detect subtelomeric abnormalities in children with severe mental retardation. Our results also provide evidence for the prevalence of the paternal origin of the rearrangements. They illustrate the potential difficulty in demonstrating the pathogenicity of previously undocumented small telomeric rearrangements. Finally our data emphasize the phenotypic variability of these subtelomeric rearrangements.

**An adjacent-2 segregation in a balanced familial t(9;15)(q32;q13) with a large centric segment.** *J. Xu, T. Heshka, M.J.M. Nowaczyk.* Dept. of Pathology & Molecular Medicine, Hamilton Regional Laboratory Medicine Program, Hamilton Health Sciences and McMaster Univ., Hamilton, ON., Canada.

Adjacent-2 segregation is rather uncommon among familial balanced translocations. It usually occurs in translocations involving the chromosomes with small short arms; mostly chromosome 9 and acrocentrics. Both centric segments are characteristically small. We report adjacent-2 segregation in a familial balanced t(9;15)(q32;q13) with a large centric segment in the derivative chromosome 9 and a small centric segment in the derivative chromosome 15. The proband is a 22-year-old mother with a maternally inherited translocation, 46,XX,t(9;15)(q32;q13). The grandmother had a history of pregnancy with an unbalanced karyotype 47,XY,+der(15)t(9;15). Our patient has had three recognized pregnancies. The first pregnancy was lost at 12 weeks gestation. Analysis of products of conception indicated the fetus had a karyotype of 46,XY,+der(9)t(9;15)mat,-15. The second pregnancy was lost at 12 weeks gestation, but was not cytogenetically investigated. The third pregnancy was terminated at 20 weeks gestation because the fetus was found by amniocentesis to also have a karyotype of 46,XX,+der(9)t(9;15)mat,-15. This karyotype was unbalanced, with trisomy for 9pter-q32 and monosomy for 15pter-q13. The abnormal karyotype was probably a result of adjacent-2 segregation. This finding indicates that this adjacent-2 segregation product is compatible with fetal life to at least mid-gestation. This is hardly surprising, considering that a full-blown trisomy of entire chromosome 9 has been documented in liveborn cases. The deficiency of the centric segment 15 is unlikely to be lethal in fetal development, because of its small genetic content. Based on the grandmother's history of pregnancy with 47,XY,+der(15), it is also possible that the abnormal pregnancies in our patient might have resulted from the loss of the der(15) from an original trisomy gamete with der(9) plus der(15), originating from a 3:1 segregation. Accumulation of more cases and further molecular investigations should help us to better understand the mode of segregation for this (or similar) translocation and its clinical implications.



**Non-acrocentric satellited chromosomes. A review.** *C. Sarri, J. Gyftodimou, M. Grigoriadou, E. Pandelia, S. Kalogirou, A. Stefanidou, M.B. Petersen.* Dept Genetics, Inst of Child Health, Athens, Greece.

Translocations involving satellites with or without the nucleolar organiser region (NOR) and the terminal region of a non-acrocentric chromosome arm result in satellited non-acrocentric chromosomes. In our Department, we have found only three cases with non-acrocentric satellited chromosomes in a total of about 7,000 karyotypes (0.04%). The three cases were 2qs, 4ps and 10qs.

Several such reports exist in the literature. These unique chromosomes presumably occur secondary to a translocation with an acrocentric chromosome. Generally, balanced carriers of such translocations are not affected, but individuals with unbalanced segregation products exhibit diverse symptoms because of partial trisomy or monosomy. Males with a Yqs chromosome generally are not affected by the secondary deletion of Yq13 (13 cases at least), because this region of the Y does not appear to contain expressed genes. There are many additional reports of satellited non-acrocentrics, i.e. 1p (two cases), 1q (two cases), 2p (one case), 2q (seven cases), 3q (one case), 4p (one case), 4q (7 cases), 9p (one case), 9q (one case), 10q (one case), 12p (one case), 18p (one case), Yp (3 cases), and Yq (at least 13 cases), in which carriers are not adversely affected even though the reciprocal translocation chromosome is absent. In other reports the carriers of a satellited non-acrocentric chromosome are affected because of proved or possible loss of genetic material. Such cases have been described concerning chromosomes 1p (two cases), 1q (one case), 4p (four cases), 4q (two cases), 5p (one case), 6q (one case), 9q (one case), 10p (one case), Xp (one case), and Yq (one case). There are some other reports of satellited non-acrocentric chromosomes of unknown phenotype involving chromosomes 1q (two cases), 2q (one case), 7q (one case), 9q (one case), and 12q (one case).

In situ hybridization with subtelomeric probes and molecular analysis are necessary to clarify the molecular event in order to give a proper genetic counseling to the families.

**Polymorphisms in genes involved in folate metabolism are not maternal risk factors for Down syndrome.** *M.B. Petersen<sup>1</sup>, M. Grigoriadou<sup>1</sup>, M. Mikkelsen<sup>2</sup>*. 1) Genetics Dept, Inst Child Hlth, Athens, Greece; 2) Dept Med Genet, J.F. Kennedy Inst, Glostrup, Denmark.

Trisomy 21 is the most common chromosome abnormality in liveborns and is usually the result of nondisjunction of chromosome 21 in meiosis in oogenesis. There is still a lack of understanding of the cellular and molecular mechanisms underlying nondisjunction. Two recent studies suggested that abnormal folate metabolism and polymorphisms in genes involved in folate metabolism may be maternal risk factors for DS (*Am J Clin Nutr* 1999;70:495-501 and *Am J Hum Genet* 2000;67:623-30). Significantly higher frequencies of the 677C>T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene and the 66A>G polymorphism in the methionine synthase reductase (MTRR) gene were found in mothers of children with DS than in control mothers. The studies suggested potential opportunities to improve public health strategies for the primary prevention of DS. We therefore analyzed the MTHFR 677C>T and MTRR 66A>G polymorphisms in a population-based study of DS in Denmark, where the origin of nondisjunction was determined by DNA microsatellite analysis. The material consisted of 181 mothers of children with DS (non-mosaic free trisomy 21) with origin of nondisjunction in maternal meiosis I or II. The frequency of the 677T allele in the DS mothers (27.7%) was not significantly different from the frequency in the fathers (29.8%) and not significantly different from the frequency in Danish controls (29.0%, n=1,084). There was no significant difference in the frequency of the 66G allele between mothers (55.3%) and fathers (53.7%). Also, there was no significant difference in the frequency of the two mutant alleles between mothers with meiosis I errors (n=126) and mothers with meiosis II errors (n=55). Furthermore, the frequency of combined MTHFR and MTRR homozygous mutant genotypes in the mothers (2/174) was not significantly different from the frequency in the fathers (3/171). We conclude that the common MTHFR 677C>T and MTRR 66A>G mutations are not likely to be maternal risk factors for DS and that the previously published findings were probably due to bias caused by population admixture.

**Centromere specific multicolor-color FISH (cenM-FISH) is a versatile method for the characterization of small SMC - case report.** *H. Starke<sup>1</sup>, A. Nietzel<sup>1</sup>, F. v. Eggeling<sup>1</sup>, A. Heller<sup>1</sup>, M. Rocchi<sup>2</sup>, B. Mitulla<sup>3</sup>, V. Beensen<sup>1</sup>, U. Claussen<sup>1</sup>, T. Liehr<sup>1</sup>.* 1) Inst. of Human Genetics and Anthropology, Jena, Germany; 2) Inst. of Genetics, Bari, Italy; 3) Central Clinic, Genetic Counselling, Suhl, Germany.

Stable small supernumerary marker chromosomes (SMC) often present without molecular cytogenetically detectable euchromatin and are uneasy to characterize in standard cytogenetic approaches. Microdissection and reverse painting could be used for their characterization. However, this technique is used more reasonably for larger markers with more euchromatin. Recently, we developed a probe set using all human centromeric probes labeled in different colors, allowing the simultaneous characterization and identification of all chromosomes by their centromeric region (Nietzel et al., *Hum Genet* 2001, 108:199-204). The technique, called cenM-FISH has been applied in the following case: Cytogenetic diagnostic was done in a newborn boy who presented with typical features of Down syndrome like hypertelorism, simian crease and hypomotility. The suspicion Down syndrome was confirmed in cytogenetics as a free trisomy 21. Additionally a small SMC was detected in 28/35 analyzed metaphase spreads (karyotype: 48,XY,+21,+mar). CenM-FISH revealed, that the small SMC was a derivative chromosome 4. As it was not stained by a whole chromosome painting probe for chromosome 4 it was described as a der(4)(p11q11). The karyotypes of the parents were inconspicuous, thus, two de novo events must have taken place to lead to the detected numerical aberrations. Uniparental disomy (UPD) 4 of the inconspicuous chromosomes 4 has been excluded for whole chromosome 4 apart from a small region in 4pter. In summary, the cenM-FISH technique is a very useful approach for the one step identification of all human chromosomes by their centromeres. Molecular genetic studies to exclude UPD should always be done after determination of SMC's origin. Acknowledgments: This work was supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the EU (ICA2-Ct-2000-10012 and QLRT-1999-31590) and the Wilhelm Sander-Stiftung.

**A prospective (three years) cytogenetical study of 114 children with congenital anomalies submitted to necropsy died between 0 to 14 years old.** *J.M. Pina-Neto<sup>1</sup>, D. Ortolan<sup>1</sup>, H.H.L Fernandez<sup>1</sup>, L.F. Mazzucatto<sup>1</sup>, L.C. Peres<sup>2</sup>.* 1) Dept Genetics, Fac Medicine Ribeirao Preto, Sao Paulo, Brazil; 2) Dept Pathology, Fac Medicine Ribeirao Preto, Sao Paulo, Brazil.

The objectives of this study were to establish an adequate cytogenetics methodology for necropsy studies and to determine the frequency and characteristics of chromosomal aberrations in children with congenital anomalies, who died in pediatric ages (0 to 14 years). In the period of the present study (November, 1996 to November, 1999) 832 pediatric necropsies were made at University Hospital. From them, 229 children (27.5%) had congenital anomalies and 144 had indications for a karyotype study. Culture success was obtained in 79.2% (114) of these cases. Chromosomal anomalies were observed in 29 children, which represents 3.5% of total sample (29/832), 12.6% among anomalous children (29/229) and 25.4% of the children karyotyped (29/114). From the 29 chromosomally abnormal children, 27 died within the first year (25% were stillbirth). After a complete clinical-genetic evaluation of the children with congenital anomalies, it was possible to detect 8 children with multiple congenital anomalies without a cytogenetic study, in which only in one of them was made a hypothesis of chromosomal disorder. The 29 cases of chromosomal disorder represented 20.1% within the etiologic factors involved in this sample. It was detected trisomy 18 (10 cases), trisomy 21 (9 cases), trisomy 13 (4 cases), trisomy 9 (1 case), triploidy 69,XXX (1 case) and 4 cases of structural aberrations (1 with Robertsonian balanced translocation 14;21; 1 with a monosomy 5p; 1 with a partial monosomy 21 and 1 with a double partial trisomy 9pter-q2 and distal 16q). The higher culture successes were obtained with fascia of abdominal rectus muscle (75.5%), followed by skin (69.5%), blood (58.7%) and kidney (33.3%). There was a clear effect of time between death and beginning of culture in the culture success rate.

**Complex Behavioural disorder in a patient with ring chromosome 10(p15;q26).** *P. Strisciuglio<sup>1</sup>, M.A. Iembo<sup>1</sup>, R. Marotta<sup>3</sup>, M.T. Moricca<sup>1</sup>, E. Rossi<sup>2</sup>, D. Concolino<sup>1</sup>.* 1) Dept Pediatrics, Univ Catanzaro, Catanzaro, Italy; 2) Dept of Biology and Medical Genetics Univ Pavia, ITALY; 3) Dept of Psychiatry, Univ Catanzaro, Italy.

Ring chromosomes have been described and are usually associated with growth and mental retardation when autosomes are involved. Only few patients with ring chromosome 10 have been reported. According to many studies that have reported association between chromosome deletion syndromes and neuropsychiatric disorders, we describe a patient with ring and deletion of chromosome 10 presenting severe psychiatric and behavioural problems. The patient, a male, was the fifth child of healthy and unrelated parents. The family history, the pregnancy and the delivery were unremarkable. During childhood there were severe feeding difficulties with progressive growth retardation. Psychomotor delay associated with comportamental abnormalities were referred during the infancy. Bilateral cryptorchidism was surgically corrected at 13 years of age. Physical examination at the age of 32 years demonstrated a low weight, a short stature, microcephaly, triangular face, pectus excavatum, micropenis normal sexual development. The neuropsychiatric examination showed mental retardation and mood disorders, moderate anxiety and personality problems. Plasma ammonia levels and acido-base status, plasma and urinary aminoacid evaluation were normal. Brain axial computerized tomography, ophthalmologic examination, echocardiography and abdominal ecography were normal. Chromosome analysis was performed on QFQ and GTG banded metaphases from synchronised peripheral lymphocyte cultures using standard procedure. Proband's karyotype was interpreted as 46,XY,ring 10(p15q26) Ring breakpoints were defined at YAC level by fluorescent in situ hybridization (FISH). The clinical features of our patient have been described in other cases of monosomy 10qter. According to the recent evidences suggesting a greater incidence of psychiatric and behavioural problems in children and adults with chromosome deletions, we suggest that the complex behavioural disturbances in our patient may be a primary feature of this chromosomal abnormality.

**Interstitial deletion of chromosome 21q.** *S. Sastry*<sup>1</sup>, *I.K. Gadi*<sup>2</sup>, *P. Papenhausen*<sup>2</sup>, *L. Mehta*<sup>1</sup>. 1) Dept. of Pediatrics, Div. of Medical Genetics, North Shore University Hospital, Manhasset, NY; 2) Laboratory Corporation of America.

Partial monosomy 21q is reported in association with unbalanced chromosome translocations, ring chromosome 21 and chromosome 21 deletions. We report a 3.5 yr. old girl evaluated for moderate developmental delays. Growth parameters were normal. Dysmorphic features consisted of flat nasal bridge, small nose, short palpebral fissures, mild hypertelorism, thin upper lip, posteriorly rotated ears with small lobules, abnormal palmar creases and overlapping toes. She had a history of infantile colic and poor weight gain. She was nearsighted and had astigmatism. Hearing, EEG and head imaging were normal. Cardiac evaluation and renal sonogram were normal. Blood chromosome analysis showed a chromosome 21 deletion, 46,XX,del(21)(q11.2-q22.1). A FISH whole chromosome 21 DNA probe study painted both homologues completely with no evidence of a balancing insertion. Parental chromosomes were normal. Interstitial deletions of 21q are rare; only 7 reports have been ascertained where the abnormality was not associated with other chromosome imbalance. Most cases were reported without FISH or molecular studies. Dysmorphic features and developmental delays are non-specific in these patients. The phenotype can be mild and one patient with normal intelligence was reported (Korenberg,1991). A possible critical region for growth and mental retardation, heart defects, facial dysmorphisms, arthrogyriposis and hypertonia has been suggested between bands 21q21-21q22.1 (genes APP to SOD1)(Chettouh,1995). Holoprosencephaly was present in one patient with del 21q22.3(Estabrooks,1990).It is suggested that deletions distal to 21q22.2 generally result in more severe abnormalities. Our patient presents with a fairly mild phenotype and a deletion proximal to the q22.2 band. Further molecular studies in such cases can provide more precise phenotype/genotype correlations.

**Duplication of chromosome 15q14q15 associated with short stature, mental retardation, speech problems and atypical body fat distribution.** *M. Weickert, M. Adamowicz, C. Baldermann, H. Thiele, U. Hehr, I. Hansmann.* Institut fuer Humangenetik und Medizinische Biologie, Halle/Saale, Germany.

Duplications of the proximal segment of maternal chromosome 15 including PWS/AS critical region has been described in several probands with a complex phenotype including developmental delay, speech and learning difficulties as well as autistic behaviour. Here we present a unique patient with a maternal duplication for 15q. The propositus is the only child of clinically normal unrelated parents. Pregnancy was complicated by placental insufficiency and IUGR. Two days after spontaneous delivery a VSD was observed. At the age of 7 1/2 years the boy revealed short stature below the 3rd percentile, developmental delay, dysmorphic facial features, feeding, speech and behavioral problems and a female-like body fat distribution. Karyotyping revealed additional bands within 18q. Analysis of the mother identified her as a carrier of a translocation 46,XX,ins(15;18)(q1?q15;q11.2). Painting for maternal chromosome 15 labeled both chromosomes 15 and a proximal 18q segment. A chromosome 18 paint labeled only the normal 18 and the der(18), respectively. No signal was present on the der(15), confirming an insertion of chromosome 15 segment into chromosome 18. FISH using SNRP (15q11.2) and PML (15q22) probe (Vysis) placed the proximal breakpoint telomeric to PWS/AS and the distal breakpoint centromeric to PWL locus. Analysis of 8 microsatellite markers representing segment 15q13-q15 revealed no evidence for duplication of any maternal allele. To further narrow down the duplication FISH was performed to metaphases of the proband using 8 YACs. According to signals obtained at the normal chromosome 15 and the der(18) the proximal boundary of the duplication is flanked by YAC 879f03 and the distal boundary by YAC 900d8. Two YACs, i.e. 850d6 and 876c4 are located within the duplication. Due to a significantly reduced signal of YAC 876c4 (approx. 890kb) on the der(18) chromosome we assume that the telomeric breakpoint maps within the template for that YAC at 15q15. A maternal dosage effect of duplicated genes within 15q14q15 is considered to be responsible for the phenotype.

**Prenatal diagnosis of an unusual translocation.** *G.S. Sekhon<sup>1</sup>, S. Jalal<sup>2</sup>, A. Salvador<sup>3</sup>, K. Thompson<sup>1</sup>, E. Johnson<sup>1</sup>, C. Corsi<sup>1</sup>.* 1) Div Clinical Gen, Waisman Ctr, Univ Wisconsin, Madison, WI; 2) Cytogenetics Lab, Mayo Clinic, Rochester, MN; 3) St. Johns Mercy Medical Center, St. Louis, MO.

Cytogenetic analysis of amniocytes obtained at 15.3 weeks of gestation revealed a female karyotype with an abnormal banding pattern of 19p terminus. There seemed to be extra euchromatic material on the short arm of chromosome 19 and it was thought that the extra material was either from 7q or 5q by G-banded analysis. Parental cytogenetic studies revealed a similar anomaly in the phenotypically normal father. To clarify this, FISH studies were performed. The individual chromosome painting probes and M FISH did not reveal the origin of the extra material on 19p terminus. FISH analysis on the amniotic fluid and on the father using Vysis subtelomeric probes 7 and 19 consisting of p and q terminal probes, showed only a signal on the p arm of one chromosome 7, with the 7q signal relocated distal to the 19p subtelomere signal on the derivative 19 chromosome. The 19p subtelomeric unique sequence DNA was retained on the derivative 19 chromosome, indicating a more distal breakpoint. Further analysis with BAC FISH is in progress to delineate the extent of the submicroscopic translocation. These findings demonstrate 1) The size of genomic material translocated from the chromosome 7 homologue was too small to be detected by chromosome painting probe or M FISH, 2) subtelomeric FISH analysis is important in delineating structural rearrangements when prometaphase analysis is questionable, but subtelomeric translocations may occur that will be undetected by subtelomeric specific FISH probes, 3) there is a need for knowing the gap size between subtelomere clones and the distal sequences, and 4) despite new technologies counseling dilemmas persist.



**Molecular cytogenetic characterization of a pericentric inversion, inv(22)(p13q13.1).** *G.V.N. Velagaleti<sup>1</sup>, C.A. Jesurun<sup>2</sup>, L.H. Lockhart<sup>1</sup>, V.S. Tonk<sup>3</sup>*. 1) Department of Pediatrics, University of Texas Medical Branch, Galveston, TX; 2) Department of Pediatrics, Texas Tech University, El Paso, TX; 3) Department of Pediatrics, Texas Tech University, Lubbock, TX.

Pericentric inversions occur at a frequency of 0.12-0.7% in humans. Although chromosome 22 has been suggested to be preferentially involved in structural rearrangements, pericentric inversions of chromosome 22 are rare. Here we report for the first time a pericentric inversion of chromosome 22; the resulting recombinant chromosome and the phenotypic features associated with this recombinant chromosome.

A newborn infant was referred because of multiple congenital anomalies. He is the first child of a 25-year-old mother with unremarkable pregnancy history. At birth his height, weight and OFC were below the 5<sup>th</sup> percentile. He had hypertelorism, depressed nasal bridge, right cleft lip, bilateral cleft palate and small, low-set and malformed ears. Chromosome analysis showed a structurally rearranged chromosome 22 with additional material on 22p. Suspecting a duplication, parental chromosome analysis was requested. The proband's mother showed a rearranged chromosome 22 that is morphologically different from the child. C- and AgNOR banding studies showed a positional shift in both centromeric heterochromatin and satellite stalks. Studies with a series of FISH probes confirmed the pericentric inversion in the mother [karyotype 46,XX,inv(22)(p13q13.1)] and showed that the proband has a recombinant chromosome 22 resulting in partial duplication of the distal 22q13.2-qter region [karyotype 46,XY,rec(22)dup(22q)inv(22)(p13q13.1)].

Our proband with distal 22q13 duplication has several features common in patients with larger duplications extending to proximal 13q and patients with complete trisomy 22. Thus, it appears that the distal trisomy for 22q13.2->qter region is sufficient to result in complete trisomy 22 phenotype. Our case illustrates the efficacy of molecular cytogenetics in delineating subtle chromosome rearrangements which would not have been accurately characterized using conventional methods.

**Cytogenetic and molecular studies on an unusual partial trisomy 13 recombinant resulting from a maternal rearranged chromosome 13.** *N. Qin<sup>1</sup>, A. Fujimoto<sup>2</sup>, A. Anguiano<sup>1</sup>, Q. Xiao<sup>1</sup>, J.-J. Chen<sup>3</sup>, Y. Wang<sup>4</sup>, C. Tuck-Muller<sup>4</sup>, T.-J. Chen<sup>4</sup>.* 1) Dept of Cytogenetics, Quest Diagnostics' Nicholes Institute, San Juan Capistrano, CA; 2) Keck School of Medicine, University of South California, Los Angeles, CA; 3) Dept. of Medicine, University of Michigan, Ann Arbor, MI; 4) Dept. of Medical Genetics, University of South Alabama, Mobile, AL.

We describe an 11 month-old boy born at 39-week gestation to a 22 year-old gravida 2 para I phenotypically normal mother. At birth, he weighed 2420 gm (3-10th centile) and had a broad nasal bridge, micrognathia, neonatal teeth and cryptorchidism. No abnormality was found in the brain, heart and kidneys by ultrasound examinations. At 11 months, he weighed 7.7 kg (<5th centile), and measured 72 cm in length (10th centile) and 44 cm in OFC (<5th centile). He had social smiles at 3 months and rolled over at 9 months. Karyotype of peripheral blood lymphocytes showed an additional genetic material attached to the short arm of one of the chromosome 13. FISH assays for chromosome 13 (whole chromosome paint, centromere, RB-1 mapping to 13q14, and D13S585 at 13q32q33) identified it as an unusual partially duplicated chromosome 13. FISH results also indicated that there was a duplication of the RB-1 locus, but only a single D13S585 locus on the derivative chromosome 13. Father's karyotype was normal, while the mother had an apparently balanced, complex abnormality of chromosome 13 with a rearrangement of the centromere, RB-1, and D13S585 loci. CGH and high density (5-cm resolution) short tandem repeat (STR) genotyping are in progress to elucidate the location and size of the duplication on the derived chromosome 13 of the proband. In summary, we identified a rare duplication that resulted in nearly complete trisomy 13, but without a typical trisomy 13 phenotype. The mechanism of rearrangement and the segregation pattern during meiosis have been studied. The correlation of genotype and phenotype will be also discussed.

**A complex rearrangement in a mother simplified into a single balanced translocation in her fetus.** *F. Tihy<sup>1</sup>, N. Lemieux<sup>2</sup>, E. Lemyre<sup>1</sup>*. 1) Dept Genetics, Hosp Ste-Justine, Montreal, PQ., Canada; 2) Dept Pathology, Hosp Ste-Justine, Montreal, PQ., Canada.

An amniocentesis was performed for advanced maternal age on a woman with mild mental retardation, short stature, facial dysmorphism, and hydronephrosis. A sample of the mother's blood was taken at the same time for karyotyping. The fetal chromosomes showed an abnormal short arm of chromosome 20, which was analyzed by FISH. Subtelomeric probes from chromosome 20 showed a balanced translocation  $t(16;20)(q23;p11.2)$ . FISH with the subtelomeric probes of chromosome 16 confirmed this result.

Surprisingly, the mother's karyotype showed a complex chromosomal rearrangement (CCR):  $46,XX,t(5;16)(q11.2;q12),t(16;20)(q23;p11.2)$ . The derivative chromosome 5 was composed of short arm and pericentromeric region on the long arm of 5, proximal long arm of chromosome 16 and distal short arm of chromosome 20.

The transmission to the fetus of a simpler balanced rearrangement involving half of the rearrangements seen on the mother  $der(5)$  is the first reported to our knowledge. Such a phenomenon can be explained by two hypotheses. Meiotic recombination between the normal 16 and  $der(5)$  would result into a recombinant chromosome containing chromosome 16 and part of chromosome 20p as seen in the fetus. The second hypothesis involves germline mosaicism in the mother for  $46,XX,t(16;20)/46,XX,t(5;16),t(16;20)$ . However, all the lymphocytes analyzed showed the complex rearrangement.

The baby was born at 33 weeks with intra-uterine growth retardation, facial dysmorphism and hydronephrosis.

**Partial 4p and 5p inverted duplications are constantly associated with cryptic terminal deletions.** *M. Zollino<sup>1</sup>, M. Petersen<sup>2</sup>, L. Bortotto<sup>4</sup>, L. Memo<sup>3</sup>, G. Zampino<sup>5</sup>, Y. Gyftodimou<sup>2</sup>, C. Sarri<sup>2</sup>, G. Neri<sup>1</sup>.* 1) Genetica Medica, Univ Cattolica, Rome, Rome, Italy; 2) Institute of Child health "Aghia Sophia" Children's Hospital Athens, Grecia; 3) Patologia Neonatale ospedale Regionale Treviso, Treviso, Italy; 4) Genetica Medica Az. Ospedaliera S.Maria della Misericordia Udine, Italy; 5) Istituto di Pediatria, Univ Cattolica Rome, Rome, Italy.

A partial 4p duplication was detected by conventional chromosome analysis in three patients presenting with a Wolf-Hirschhorn syndrome (WHS) phenotype. One of these patients was already reported (Zollino et al, 1999). By FISH, the duplication was demonstrated to be inverted. On clinical evidence, a concomitant 4p deletion, encompassing the WHS critical region, was detected by FISH in each occasion. A different size of both the duplication and the deletion was observed both cytogenetically and molecularly in the three patients, the proximal breakpoint of the duplication being at bands p15.1, p15.2 and p16.1, the duplication-deletion boundary being at bands p16.2, p16.1 and p16.3. respectively. Clinical manifestations were only consistent with a WHS phenotype, and were according to the size of the deletion. The only sign consistent with the phenotype of partial 4p trisomy syndrome was hypoplastic corpus callosum, that was associated with the larger duplications (two patients). A similar rearrangement, consisting of inverted partial duplication on 5p and cryptic deletion encompassing the cri du chat syndrome locus, was diagnosed in a newborn with multiple congenital malformations/ dysmorphisms, including the cri du chat syndrome clinical manifestations. From these observations, we can infer that 1) inverted duplications affecting the distal half of 4p and 5p chromosome arms are always associated with a terminal deletion, as a consequence of a common meiotic rearrangement; 2) the presence of an undiagnosed deletion is likely to be responsible for some phenotypic inconsistencies so far observed among apparently pure partial 4p trisomies; 3) such double chromosome imbalance identify a specific subset of WHS and cri du chat syndrome patients.

**Complex chromosomal rearrangements - 7 new cases and a new classification system based on mechanism during cell division.** *S.M. Zneimer, J.C. Kelly, S.D. Stewart.* Cytogenetics Laboratory, Quest Diagnostics, Van Nuys, CA.

We report 7 cases of complex chromosomal rearrangements (CCRs) in a series of 6000 post-natal chromosome studies: Case 1: 46,XX,der(2)t(2;3)(p15;q27)inv(2)(q14q23) Case 2: 46,XY,der(12)inv(12)(q21.3q24.1)t(1;12)(q42.3;q24.3) Case 3: 46,XX,der(5)inv(5)(p13p15.1)t(5;8)(q23.2;q22.1)/46,XX Case 4: 46,XY,der(7)t(7;13)(p11.2;q14.3)t(7;21)(q11.2;q22.3) Case 5: 46,XY,t(1;7;14)(q32.3;p21.2;q21.2) Case 6: 46,X,ins(X;8)(p22.1;p23.1p23.3) Case 7: 46,XY,der(18) inv(18)(p11.2q12.2) t(15;18)(q21.2;p11.32) Four of these cases are of phenotypically normal individuals with reproductive problems, and 3 of these cases are children with phenotypic problems. Four of these cases required fluorescence in situ hybridization to define the breakpoints involved. There are various classifications of CCRs described in the literature; however, none of the classifications help define the cases of CCRs reported here. Along with a description of the genotype of these cases and their correlation with phenotype, we propose a new classification system to better define CCRs. It is the underlying mechanism occurring during cell division that defines the structure of chromosomes and this classification system reflects those differences. The classification is divided into 3 categories differentiating the number of breaks per chromosome that ultimately give rise to specific types of chromosome aberrations: 1) At least three breaks in a single chromosome with at least one break in a second chromosome (e.g. at least one chromosome with an inversion and a translocation event that involves at least a second chromosome). 2) Two breaks in a single chromosome with a single break in a second chromosome (e.g. an insertion of genetic material from one chromosome into a second chromosome). 3) A single break in three or more chromosomes (e.g. three or more chromosomes involved in a related translocation event, or 2 or more unrelated translocation events involving at least 3 chromosomes). This is the first classification system based on mechanisms during cell division and may be helpful in defining differences between complex cytogenetic aberrations.

**Identification of the sex determining region (SRY) gene in 46,XX males.** *J.G. Pappas<sup>1</sup>, M.J. Macera<sup>2</sup>, V. Mizhiritskaya<sup>2</sup>, A. Babu<sup>2</sup>.* 1) Clinical Genetic Services, New York University, School of Medicine, NY., NY; 2) Dept. of Molecular Medicine and Genetics, Wyckoff Heights Medical Center, Brooklyn, NY.

The sex determining region (SRY) gene, located at Yp11.2, regulates the expression of male specific genes by changing chromatin structure and creating new binding sites for other transcription factors [Giese et al 1992]. Cases of sex reversal 46,XX males, generally are discovered because of infertility; with the exception of sterility, there are generally no phenotypic differences with 46,XY males. The X and Y chromosomes pair and recombine briefly during male meiosis in two homologous regions the pseudoautosomal regions (PAR) located at the distal p and q arms of the X and Y. Abnormal exchanges of the X-Y can lead to transfer of Y material, including the SRY gene, to the p arm of the X chromosome. Receipt of this chromosome by a 46,XX individual will confer a male phenotype. To date, 80% of the 46,XX males investigated are positive for the presence of the SRY gene. The PRKY and PRKY are two homologous genes on the Y and X chromosomes respectively. The PRKY gene is located within an inversion polymorphism on the p arm of the Y chromosome. Sixty percent of normal 46,XY males showed the inversion polymorphism in one orientation, where as less than 10 percent of 46,XX SRY+ males showed the same orientation, suggesting illegitimate recombination between these two homologous genes as a possible cause for the formation of 46,XX, SRY+ males [Tar et al 1995, Aleck et al 1999, McElreavey and Fellous 1999]. We were referred two phenotypically normal males due to infertility. The clinical examination was normal except for micro-orchidism, which has been described [Margarit et al 1998]. Chromosomal analysis with GTG banding revealed a 46,XX karyotype in both cases. FISH analysis with a cocktail probe for the SRY gene and the X centromere (Vysis) identified the presence of the SRY gene in both cases. The karyotypes were revised to 46,XX.ish t(X;Y)(p22.3;p11.3)(DXZ1+,SRY+). Studies are under way to determine the orientation of the inversion polymorphism in the paternal Y chromosomes. It is through such studies that a clearer understanding of sex determination can be obtained.

**Two Cases with Xp Deletions: Characterization Using Subtelomeric FISH Probes.** *D.I. Quigley, K.A. Kaiser-Rogers, M.I. Roche, C.M. Powell.* Depts of Pathology and Pediatrics, Univ North Carolina, Chapel Hill, NC.

Chromosome specific subtelomeric FISH probes are useful in the ascertainment of cryptic rearrangements involving chromosome ends. These probes may also be beneficial for identification of complex rearrangements that appear, by G-banding, to be simple cytogenetic aberrations. We report two cases in which subtelomeric FISH probes (Vysis ToTelVysion) identified a cryptic deletion and a complex rearrangement of the X chromosome, respectively. The first case is a female patient with mild mental retardation and multiple minor anomalies who was approximately 14 weeks pregnant at the time of her referral. Karyotype analysis, fragile X testing and FISH for DiGeorge syndrome yielded normal results. A subtelomeric FISH assay revealed a deletion of the Xp subtelomeric region. Additional FISH studies indicated no deletion of the steroid sulfatase (STS) or Kallmann genes. Thus the Xp deletion identified in this patient lies distal to the STS gene and is cryptic. Since the Xp subtelomeric region is reportedly polymorphic, we do not know if the deletion in this patient represents a polymorphism without clinical significance. Parental karyotypes have been requested. The second case is a female who was referred for short stature, hypothyroidism, renal problems and a history of failure to thrive. Cytogenetic analysis revealed a large Xp deletion, 46,X,del(X)(p11.2?3). FISH for subtelomeric sequences was performed to determine if the deleted X chromosome contains material from another chromosome. FISH analysis revealed hybridization of the Xq, rather than the Xp, subtelomeric probe to the p-arm of the deleted X chromosome. None of the subtelomeric probes hybridized to the q-arm of the deleted X chromosome. This result suggests that the abnormal X chromosome observed in this patient carries a terminal deletion of the p-arm as well as a large pericentric inversion. Our studies underscore the versatility and value of the subtelomeric FISH probes. These probes are not only useful in indentifying cryptic rearrangements, but also in characterizing seemingly simple cytogenetic aberrations.

**Delineation of phenotype resulting from an unbalanced complex chromosomal rearrangement t(6;15;7)**

**(p11.2;q26.1;p15.3).** *S.Kim. Tan<sup>1</sup>, Y.S. Choy<sup>1</sup>, A. Othman<sup>1</sup>, S.K. Ten<sup>2</sup>, M.Z. Norzila<sup>1</sup>.* 1) Cytogenetics, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia; 2) Cytogenetics, Institute of Medical Research, Kuala Lumpur, Malaysia.

Complex chromosomal rearrangements (CCR) resulting from three way translocation are rare. Most of the three way CCRs are usually familial and transmitted through the mother. Here we describe two siblings with unbalanced karyotype inherited from a 28 year old G4P2A2 mother who has an apparently balanced three way translocation 46,XX,t(6;15;7)(p11.2;q26.1;p15.3) and relatively normal phenotype except for preauricular pits. The CCR were identified using high resolution karyotyping and fluorescence in situ hybridization/whole chromosomal painting. Both the siblings had partial trisomy 7pter-15.3 and a deletion of 15q26.1-qter. Both of them have proportionate short stature and dysmorphic facies characterized by macrocephaly and prominent forehead, large anterior fontanel with delay closure, hypertelorism, strabismus, short palpebral fissure, preauricular ear pits with low set ears, micrognathia and short neck. There were prominent fetal pads of the finger tips. Both had umbilical hernia and single café au lait spot. One of them had patent ductus arteriosus and pulmonary stenosis while the other had hyperactive airway disease. Both had moderate degree of global developmental delay, generalized tonic-clonic epilepsy, central hypotonia and incoordination. MRI of the brain revealed dysgenetic corpus callosum with septum pellucidum, increase extra-axial space and slightly hypoplastic cerebellum. Similar features were found in one of their uncle and one of their cousin brothers on maternal side of family, the mother of whom also had preauricular ear pits.



**Chromosomal instability in a family with a paternal  $der(1)inv(1)(q25.1q31.1)inv(1)(q32.3q42.3)$ .** *D.J. Tomkins, N.J. Leonard.* Medical Genetics, University of Alberta and Stollery Children's Health Centre Edmonton, Alberta, Canada.

A family with unusual chromosomal instability was identified through a proband with global developmental delay, failure to thrive and mild dysmorphism. She was found to have an abnormal chromosome 16 with extra chromosomal material in the long arm. Her mother, who had mild dysmorphism and learning difficulties, carried the same chromosomal rearrangement, but it was not found in either of the mother's parents. However, the mother's father had an abnormal chromosome 1 which appeared to be balanced. He passed this chromosome on to a phenotypically normal daughter, but another daughter had an abnormal chromosome 1 with extra chromosomal material in the long arm which she passed on to two sons. These three individuals had minor dysmorphic features and developmental delay. All of the individuals with normal karyotypes had normal phenotypes.

FISH with a multiprobe device for all whole chromosome paints (Cytocell OctoChrome) showed that the unbalanced rearrangement of chromosome 1 was a duplication of chromosome 1 material. The paternal chromosome 1 was also entirely painted with a whole chromosome 1 paint. Therefore, the balanced paternal chromosome 1 rearrangement was interpreted as a derived chromosome 1 with two inversions in the long arm:  $der(1)inv(1)(q25.1q31.1)inv(1)(q32.3q42.3)$ . The abnormal karyotype in his daughter and two grandsons was an inverted duplication of chromosome 1 q32.3 to q42.3. The chromosome 16 rearrangement was also an inverted duplication, of chromosome 16 q12.1 to q21. The phenotype-karyotype correlations confirmed the interpretations of the unbalanced chromosomes as duplication chromosomes.

The occurrence of different inverted duplications in two offspring of a carrier of a balanced chromosomal rearrangement is unusual. The duplication 1q cannot be explained by simple recombination, but could have arisen by an abnormal recombination event at the site of an inversion loop formation. The duplication 16q could also have occurred by an abnormal recombination event. It is possible that an abnormality in recombination or repair led to the chromosomal instability observed in this family.

**Y chromosome abnormalities in two patients with congenital diaphragmatic hernia.** *C.M. Powell<sup>1</sup>, K.W. Rao<sup>1,2</sup>*. 1) Div Genetics & Metabolism, Department of Pediatrics, UNC-Chapel Hill, Chapel Hill, NC; 2) Department of Pathology, UNC-Chapel Hill.

Congenital diaphragmatic hernia (CDH) is a developmental field defect with causal heterogeneity. It has an incidence of 1/3500 births and approximately 1000 infants are born with it each year in the U.S. It comprises 8% of all major congenital anomalies. There is a high rate of morbidity and mortality with reported survival rates ranging from 25-83% (average 60%). Chromosomal syndromes with CDH include trisomy 13, 18 and 21, tetrasomy 12p, monosomy 1q32-42, and monosomy 15q24-26. There have been rare reports of patients with Turner syndrome and diaphragmatic hernia. Abnormalities of the Y chromosome have not been reported. We report two unrelated patients with CDH and sex chromosome mosaicism with cell lines containing a structurally abnormal Y chromosome. Patient 1 is a phenotypic male with 45,X/46,X ring Y mosaicism found in amniocytes and peripheral blood. FISH revealed the presence of SRY and DYZ2 on the ring. In addition to a left CDH, the patient had a jejunal stricture and atrial flutter. Growth and development at age 12 months were normal. Patient 2 had a left CDH, coarctation of the aorta, genital abnormality with enlarged labia majora and clitoris, a unilateral multicystic dysplastic kidney and pedal edema. Chromosome analysis of peripheral blood revealed 45,X in 103/105 cells. Two cells had 46 chromosomes with an isodicentric Y chromosome confirmed by FISH analysis using SRY. This chromosome consists of 2 copies of the Y short arm, 2 centromeres, and 2 copies of the Y proximal long arm. At age 6 months she has symmetric growth retardation, microcephaly, and global developmental delay.

Deletions of Yp or Yq are not typically associated with congenital anomalies. A structurally abnormal Y chromosome may increase the risk for CDH when combined with a 45,X cell line. Due to the risk of gonadoblastoma it is important to search for a Y chromosome in patients with 45,X, but the presence of CDH should prompt a more extensive search including FISH analysis of interphase cells to look for low-level mosaicism.

**Unexpected positioning of subtelomeric DNA probes in balanced chromosome anomalies: potential effects on chromosome pairing and clinical consequences.** *P.N. Rao<sup>1</sup>, M.J. Pettenati<sup>2</sup>, P. Mowrey<sup>3</sup>, K.M. May<sup>4</sup>*. 1) Univ California at Los Angeles,CA; 2) Wake Forest Univ School of Medicine,NC; 3) Laboratory Corporation of America,NC; 4) Emory Univ School of Medicine,GA.

Molecular cytogenetic analyses with subtelomeric DNA probes provide a finer examination of cryptic chromosome rearrangements. Balanced anomalies generally have no direct clinical implications. Routine FISH analyses using subtelomeric probes identified unexpected results in 4 of 6 normal individuals with apparently balanced chromosome anomalies and a history of miscarriages and/or abnormal outcomes. The breakpoints in one chromosome occurred at or near the telomere. Three cases were reciprocal translocations: t(9;11)(q12;q25); t(4;15)(q21;q26.3);t(11;16)(p15.5;q22); and one a paracentric inversion :inv(4)(q33q35.2). In Case 1, FISH with chromosomes 9 and 11 dual-color subtelomere probes (VYSIS) showed both 11q and 9q specific probes on the long arm of the rearranged 11. There was no evidence of the 11q probe at 9q12. Similarly, in Case 2, the rearranged 15q had both 15q and 4q subtelomere probes, while the reciprocal chromosome 4 had no subtelomere signals at 4q21. In Case 3, the rearranged 11p had signals from both 11p and 16q subtelomeric probes. In each of these cases, one of the involved chromosomes lacked a subtelomere. In the paracentric inversion case, the subtelomeric 4q probe was identified within the q-arm at 4q33. Thus while the rearrangements were apparently balanced and present in normal individuals the FISH results were unexpected. Whole chromosome paint probes failed to detect this apparent imbalance. Chromosomal terminal regions are unique, rich in genes, show relatively elevated meiotic recombination, and are the first regions to pair at the onset of meiosis. So the relocation and hence chromosomal loss of a subtelomeric region could possibly disrupt chromosome pairing and have an effect on gamete production. Our serendipitous finding of an unusual relocation of subtelomeric probes in 4 cases with terminal band chromosome rearrangement regions and abnormal reproductive histories may suggest that such individuals may be at an increased risk for miscarriages and/or abnormal outcomes.

**Subtelomeric Testing: "Don't Leave Clinic Without It".** *E.H. Zackai, I.D. Krantz, J.E. Ming, K.W. Gripp, P. Kaplan, L. Bason, L. Celle, K.L. Russell, D.M. McDonald-McGinn, N.B. Spinner.* Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA.

From a multi-center study in the UK, deVries et al. (J Med Genet 38:145, 2001) identified 29 patients with a submicroscopic subtelomeric rearrangement (SSR). They suggested that significant preselection criteria for SSR studies include: IUGR and a family history of mental retardation (MR). However, 35% of their patients would have been missed if only those indicators were used for selection. They then suggested a five category scoring system to guide the selection of cases: 1) IUGR, 2) a family history of MR, 3) post-natal growth differences, 4) facial dysmorphism, and 5) congenital anomalies (with a maximum of 2 points for each category and a maximum total of 10 points). Using a minimum score of 3, all of their cases of SSR would have been pre-selected and 20% of cases with MR could have been excluded from testing. Based on these studies, we performed a retrospective review of our own SSR cases (12) at The Children's Hospital of Philadelphia utilizing the deVries checklist. The rearrangements included the following subtelomeric deletions: 1p (4), 6p, 9q, 10p, 11q (2), and 20q; and duplications of 16p and 5q. After diagnosing two patients with 1p deletion, another with a similar phenotype was targeted for 1p studies. Of note, only 1 patient had IUGR and 3 had a positive family history of MR. Utilizing the two significant preselection parameters of deVries, two-thirds of our cases would have been missed. On the deVries checklist, using a minimum score of 3, 4 of our 12 cases would have been missed. Based on these findings, it is our impression that a patient whose findings prompt a high-resolution karyotype also deserves one that rules out SSR. We advise using no preselection criteria for patients with a "chromosomal phenotype". Furthermore, as additional cases are identified and reported, specific phenotypes may emerge which could potentially lead to targeted SSR studies.

**Clinical Frequency of Chromosomal Abnormalities Observed in a Consecutive Series of Patients with Autistic Disorder.** *C.M. Wolpert<sup>1</sup>, S.L. Donnelly<sup>1</sup>, H.L. Abel<sup>1</sup>, S.A. Ravan<sup>2</sup>, H.H. Wright<sup>2</sup>, R.K. Abramson<sup>2</sup>, G.R. DeLong<sup>2</sup>, M.L. Cuccaro<sup>2</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Dept Medicine, Duke Univ Medical Ctr, Durham, N.C; 2) University of South Carolina Columbia, S.C. 29208.

Numerous reports cite the co-occurrence of AutD with various chromosome anomalies suggesting the potential of a causal relationship in a subset of cases. We examined the frequency of chromosome anomalies in 333 AutD patients consecutively ascertained for a genetic research study. These 333 AutD patients came from 99 multiplex and 127 singleton families. All the research participants had their diagnosis confirmed using the Autism Diagnostic Interview-Revised (ADI-R) and were included in this analysis if they met the strict diagnostic criteria for AutD. 32% (N=106) of the AutD individuals had had chromosome analysis done as part of their medical evaluation. Seven different chromosomal anomalies were observed in 7 independent families. The anomalies included: 18q- (3 patients); de novo, partial duplication of 7p, familial paracentric inversion of 7q, inv (7q) (q22.1-q31.2); XO; 2q- (1 patient); familial 13;14 Robertsonian translocation; and isodicentric chromosome 15 anomalies (5 patients). In one family, 4 members carried the chromosomal anomaly inv (7q) (q22.1-q31.2), but only the two male children met strict diagnostic criteria for AutD. In a second family, the Robertsonian translocation was maternally transmitted with only the son being affected. The remaining chromosomal anomalies, observed only in singleton families, were de novo occurrences in the affected individual. Here we report the clinical, developmental, and cytogenetic results of these individuals and compare them with the remaining AutD patients who had no chromosomal anomaly identified.

**A Case Report of a Complex Chromosomal Rearrangement Consisting of a Four-Way Non-Reciprocal Balanced Translocation.** *T.L. Prosen, U. Surti, W.A. Hogge.* Dept Genetics, Magee Womens Hosp, Pittsburgh, PA.

We report a case of a phenotypically normal patient with a de novo complex chromosomal rearrangement consisting of a four-way non-reciprocal balanced translocation. This 33-year old patient presented to our institution for prenatal care in her second pregnancy. Her first pregnancy had ended in an early spontaneous abortion. Her prenatal course had been uncomplicated until a routine ultrasound at 21 weeks gestation noted a fetal omphalocele. Multiple marker screening had been declined. Amniocentesis was performed and revealed an unbalanced translocation resulting in a partial trisomy 18. 46,XY,der(21)t(18;21)(q21.1;p11.2). The couple opted to terminate the pregnancy and underwent labor induction at 22 weeks gestation. The stillborn male fetus had an omphalocele, but no other gross structural abnormalities. Autopsy was declined. Maternal karyotype revealed a complex chromosomal rearrangement consisting of a four-way non-reciprocal balanced translocation. 46,XX,t(4;15;18;21)(q12;q11.2;q21.1;p11.1). Karyotypes were obtained on the parents of the patient and were found to be normal. Since that time, the patient has had two pregnancies that have both resulted in spontaneous abortion. The first of these pregnancies spontaneously aborted at 6 weeks gestation. No tissue was available for karyotyping. The second was a missed abortion identified at 9 weeks 0 days gestation. An ultrasound showed a crown rump length consistent with 7 weeks 5 days gestation without cardiac activity. Dilation and curettage was performed and tissue was sent for fetal karyotype but grew very poorly. A single cell was able to be analyzed but there was significant overlap of the chromosomes. A Y chromosome was visualized. Only one of each of the translocated chromosomes could be identified.

**Identification of a familial interstitial 13q deletion passed from an unaffected father to a daughter with cardiac malformations.** *J. Winters*<sup>1</sup>, *K. Brookhyser*<sup>2</sup>, *M. Lipson*<sup>2</sup>. 1) Genetics Department, Kaiser Permanente Northern California, San Jose; 2) Sacramento.

Deletions and duplications of euchromatic material in humans are almost always associated with anatomic and phenotypic abnormalities, developmental delays, and even reduced lifespan. However, there are reports of various gains and losses of genetic material from several chromosomes which are familial and whose carriers have no apparent problems related to the extra or missing material.

We describe a female infant delivered prematurely to a 32-year-old G5P2SAB2TAB1 woman with pregnancy-induced hypertension. The baby was intubated due to meconium and, other than fused eyelids, appeared normal at birth. On day three, she was noted to have a murmur and was diagnosed to have patent ductus arteriosus, ventricular septal defect, and atrial septal defect. Blood chromosome analysis was performed, but the chromosome morphology was extremely poor. A deletion within the long arm of chromosome 13 was suspected, and a repeat analysis confirmed it. The karyotype was determined to be 46,XX,del(13)(q21.2q22). Studies of parental blood chromosomes revealed that the 24-year-old father had an identical-appearing deletion. FISH was performed with a whole chromosome painting probe (Vysis, Inc.) for chromosome 13 to rule out an interchromosomal rearrangement. No other chromosome was found to contain chromosome 13 material, confirming the interstitial nature of this deletion. He is reportedly normal, although he carries the diagnosis of attention deficit hyperactivity disorder. The proband underwent surgery to repair her heart defects at 3 months of age and died shortly after surgery due to respiratory failure.

Cases such as this one cloud the prognosis of an individual in whom a deletion or duplication has been found. Even confirming the familial nature of the rearrangement may not assure the absence of structural anomalies or developmental delays. Possible explanations for the inconsistencies observed in this father and daughter and a review of the literature will be presented.

**Evaluation of MAPH for the determination of subtelomeric deletions.** *E.A. Sistermans<sup>1</sup>, W.M. Nillesen<sup>1</sup>, C. Van Ravenswaaij<sup>1</sup>, D. Smeets<sup>1</sup>, H.G. Brunner<sup>1</sup>, E. Hollox<sup>2</sup>, J.A.L. Armour<sup>2</sup>.* 1) Dept Human Genetics, Univ Medical Centre Nijmegen, Nijmegen, Netherlands; 2) Institute of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham, United Kingdom.

Sub-telomeric deletions are a frequent cause of mental retardation. Fluorescent in situ hybridization (FISH) is currently the most widely used technique for the detection of these deletions. Unfortunately, even when all probes are tested in parallel, this technique is time-consuming and does not allow a cost-effective analysis of the large numbers of patients that are generally sent in for routine analysis to a diagnostic service. Multiplex amplifiable probe hybridization (MAPH) is a new technique for the determination of locus copy number that does allow the analysis of large numbers of patients for tens of loci at the same time. Patient DNA is first hybridized with a set of amplifiable probes, followed by amplification of all probes using common flanking primers. Analysis of the resulting peaks, which differ in length for each probe, allows the detection of deletions and duplications. To establish the reliability of MAPH for the detection of sub-telomeric deletions, we tested the technique on a panel with 30 patients with chromosomal abnormalities that included sub-telomeric regions. First results on a limited number of patients show that all abnormalities were correctly identified. Most strikingly, a very small deletion of 17q was identified in a patient with a known duplication of this chromosome arm as detected by FISH analysis, demonstrating the presence of a deletion/duplication event.



**Small interstitial 4q21 deletion that appears to create hemizyosity for PKD2 gene (polycystic kidney disease, type II).** *M.T. Velinov<sup>1,2</sup>, J. Kupferman<sup>1</sup>, S. Barrett<sup>1</sup>, D. Rosa<sup>1</sup>, M.J. Macera<sup>3</sup>, A. Babu<sup>3</sup>, G. Kupchik<sup>1</sup>*. 1) Department of Pediatrics, Maimonides Medical Center, Brooklyn, NY; 2) Department of Cytogenetics, NYS Institute for Basic Research, Staten Island, NY; 3) Department of Molecular Medicine and Genetics, Wyckoff Heights Medical Center, Brooklyn, NY.

We report a 3-year-old boy with severe development delay and dysmorphic features: hypertelorism, large head with frontal bossing, broad flat nasal bridge, up-slanting palpebral fissures, simple external ears. The patient also had short and mildly contracted fingers with mild interdigital webbing. He was born post term, with perinatal hypoxia. The birth weight was 5 lbs and 13oz. The family history was unremarkable. He had motor and cognitive developmental delay since birth. This patient was found to have large complex cyst in his right kidney and multiple small cysts bilaterally. He underwent surgical excision of the large renal cyst. He also presented with neurological abnormalities: arachnoid cyst, muscle hypotonia and increased DTR. He had two episodes of bowel obstruction for which surgical intervention was necessary. His ophthalmological exam was unremarkable. G-banding chromosome analysis revealed an abnormal karyotype with a small interstitial deletion of the long arm of chromosome 4 with most likely breakpoints at 4q21.1 and 4q21.3 - karyotype 46,XY,del(4)(q21.1q21.3). Our patient presented with dysmorphic features, similar to the ones observed in previously reported cases with 4q21-q23 chromosomal deletions (Nowaczyk et al. Am J Med Gen, 69:400, 1997). Other previously reported phenotypic associations, with proximal 4q deletions, absent in our case include Rieger anomaly (associated with more distal deletions) and piebaldism (probably result of more proximal deletions). In contrast our patient had kidney abnormalities consistent with Adult Polycystic Kidney Disease. The gene for Adult Polycystic Kidney Disease PKD2, has been mapped to chromosome region 4q21-4q23 (MIM#173910). We suggest that our patient's hemizyosity for PKD2 explains his renal findings. We are planning further molecular studies to better define the extent of this microdeletion.

**Molecular characterization of a satellited chromosome 22 in a patient with mental retardation and no dysmorphic features.** *V.R. Pulijaal<sup>1</sup>, S.K. Gogineni<sup>1</sup>, S.J. Gross<sup>1</sup>, D. Iacoboni<sup>2</sup>, R.W. Marion<sup>3</sup>, A. Shanske<sup>3</sup>.* 1) Dept OB/GYN, Albert Einstein Col Medicine, Bronx, NY; 2) Jacobi Medical Center, Bronx, NY; 3) Dept. of Pediatrics, Montefiore Med Ctr, Bronx, NY.

Satellited nonacrocentric chromosomes are derived from the translocation of the nucleolus organizer region( NOR ) of acrocentric chromosomes and are generally considered to be of no clinical significance but there are some reported cases with variable phenotypes and reproductive outcomes. We report a 20-year-old male with hypotonia, seizures, ear anomalies, mental retardation and delay in expressive speech. High resolution chromosome studies revealed a 46,XY,22qs karyotype . Molecular characterization of satellited chromosome 22 revealed a subtelomeric deletion of 22q telomere using a 22q subtelomeric probe i.e., 46,XY,22qs.ish del(22)(q13.33)(pVYS207M-). The mother has a normal 46,XX.ish 22q13.33(pVYS207x2) chromosome complement and the father was not available for testing. Further molecular studies are in progress to investigate any genotype/phenotype correlations of the 22q subtelomeric deletion in this patient, and also to verify a possible causal role of the ribosomal gene position effects in the 22q13 region.

**Comparison of whole chromosome painting probes and telomeric region probes in identifying *de novo* chromosomal duplication.** C.W. Yu<sup>1</sup>, H-G. Bock<sup>1</sup>, F.G. Megason<sup>2</sup>, M.H. LeBlanc<sup>2</sup>, P.G. Rhodes<sup>2</sup>. 1) Dept Preventive Medicine; 2) Dept Pediatrics, Univ Mississippi Medical Ctr, Jackson, MS.

The addition chromosomal material of unknown origin, especially in the *de novo* cases, provides challenges for cytogenetic diagnosis. Identifying the extra chromosomal material would add valuable information for genetic counseling and patient treatment. We present here three cases of *de novo* chromosome duplication and compare the diagnostic value between the whole chromosome painting (wcp) probes and the telomeric (telo) region probes. **Case 1.** SM, a female newborn, referred for chromosome study because of clinical features of flat nasal bridge, unilateral hernia and signs of Down syndrome. Cytogenetic study revealed an abnormal 9 with extra chromosomal material on the short arm. WCP probe indicated that the extra material was from chromosome 9. Telo region probe identified the duplication as an inverted terminal duplication. Karyotype is 46,XY, dup(9)(p24p13). **Case 2.** BN, a male newborn, referred for chromosomal study because of MCA. Clinical features include unilateral cleft lip, cleft palate, brachycephaly, and clubfoot. G- and C-banding revealed that this patient had an extra dicentric marker chromosome. WCP probe revealed that this marker was derived from chromosome 9. Telo region probes identified the marker as an iso-9p. Karyotype is 47,XY,+idic(9)(q12). **Case 3.** KD, a 2.5-year-old girl, referred for chromosome study because of mild dysmorphism and developmental delay. Clinical features include upward slanting palpebral fissures, flat nasal bridge, short philtrum and capped teeth. G banding revealed an abnormal X chromosome with additional material on the long arm. WCP probe demonstrated the extra material was from an X chromosome. Telo region and locus specific probes revealed a duplication of Xp11.23 to Xpter and a deletion of Xq27.1 to Xqter.

For the study of *de novo* chromosome duplication, the telomeric probes seem to provide more information than the wcp probes towards chromosome identification. With the addition of proper unisequence DNA probe(s), the *de novo* duplicated segment can be properly identified.

**Pure Partial Trisomy of Chromosome 2q22-q23 Secondary to a Paternally Inherited Direct Insertion.** A. Sajoo, M.D. Speevak, S.A. Farrell. Genetics, The Credit Valley Hospital, ON, Canada.

We report the smallest duplication of 2q to date resulting in pure partial trisomy in a male infant who presented at 2 weeks with minor congenital anomalies and facial dysmorphism. BW was 50<sup>th</sup> centile, height and OFC were below the 10<sup>th</sup>. The infant had hypospadias and right foot post-axial polydactyly. Tone was normal. Developmental delay was apparent by 5 months. Echocardiogram, renal and head ultrasounds were normal. Family history was unremarkable. The infant's G-banded chromosomes showed 46,XY,der(5)ins(5;2)(q22;q22q23). This karyotype was paternally derived. Maternal chromosomes were normal. Commercially available microdissected band-specific FISH probes confirmed the direct insertion of bands 2q22-q23. Three other individuals with pure partial trisomy 2q encompassing this region have been reported, none of whom survived. All three were larger *de novo* duplications causing structural CNS and renal anomalies, and a variety of dysmorphic facial features including a prominent forehead. Complex congenital cardiac anomalies and either polydactyly or abnormal toes were noted in two cases of dup(2)(q21q33), but not in the case of dup(2)(q21q31). Common to all were low set ears and hypoplastic genitalia or hypospadias. Our case can be differentiated from the other reports by its viability, the absence of structural CNS, cardiac, and renal anomalies and the presence of a receding forehead. Ascertainment of this small insertion was through a liveborn with anomalies, which implies the recurrence risk for other carriers in this family could be up to 50% (Gardner & Sutherland, 1996). The duplicated segment in our case comprised only 0.61% of total HAL (haploid autosomal length), suggesting equal segregation ratios for balanced and unbalanced progeny. A private segregation analysis could be provide if paternal relatives are found to have this rearrangement.

**Use of M FISH, painting probes, and subtelomeric probes in refined Cytogenetic diagnosis.** *A. Salvador<sup>1</sup>, G. Sekhon<sup>2</sup>, S. Jalal<sup>3</sup>, K. Thompson<sup>2</sup>, D. Wargowski<sup>2</sup>.* 1) Dept. of Ob/Gyn, St. Johns Mercy Medical Center, St. Louis, MO; 2) Div Clinical Genetics, Waisman Center, University of Wisconsin, Madison, WI; 3) Cytogenetics Lab, Mayo Clinic, Rochester, MN.

We have utilized M FISH and subtelomeric probes along with conventional cytogenetic analysis to improve the ability of cytogenetic diagnosis. Five patients were initially detected using prometaphase G banding analysis. The first case had additional material on the short arm of chromosome 20, the second case had a duplication of the short arm of chromosome 8, the third case had additional material on the short arm of chromosome 4, the fourth case had a possible duplication or insertion in the long arm of chromosome 1, and the fifth case had a supernumerary marker chromosome. In the first case, M FISH showed that the extra euchromatic material on 20p was from chromosome 8. In the second case, M FISH showed that the duplicated material was from chromosome 8. Further analysis with subtelomeric probes showed that the 8pter probe was absent. In the third case, painting probes showed that the extra material was from chromosome 4. The fourth case by painting probe revealed a duplication of part of the long arm of chromosome 1 and the maternal karyotype showed a direct within-arm intra chromosomal insertion in chromosome 1. The fifth case of supernumerary marker chromosome was from a prenatal diagnosis. The M FISH was uninformative since the marker had no euchromatic material and this would be expected. The use of alpha and beta satellite probes showed that the marker was derived from 14 or 22. The results contributed to the counseling and the parents chose to continue the pregnancy. These strategies are particularly useful in characterization of de novo rearrangements or in cases where parental studies are not possible. These studies indicate that molecular cytogenetic studies applied in a systematic fashion can aid in the cytogenetic diagnosis and clinical management.

**Subtelomere Analysis of 270 Cases Referred for Nonspecific Idiopathic MR and/or Nonspecific Mild Dysmorphic Features.** *J.H. Tepperberg, I.K. Gadi, P.N. Mowrey, K.K. Phillips, P.F. Stone, S.K. Griffin, C.J. Bullen, P.R. Papenhausen.* Dept Cytogenetics, Laboratory Corp of America, Res Triangle Pk, NC.

Subtle subtelomere chromosome alterations are reported to occur in 7.4% of children with moderate to severe MR (Knight et al., 1999). Our center received 270 cases for subtelomere FISH testing with the majority of patients referred for nonspecific idiopathic MR and/or nonspecific mild dysmorphic features. G-band analysis was performed concurrently with or prior to the FISH analysis. Fourteen patients were found to have a terminal rearrangement (14/270 = 5.2%). Three of the 14 cases were found to have a 2qter deletion, which in all cases were shown to be familial chromosome variants. This essentially reduces the yield to 4.1% (11/270). Eight of the 11 patients had apparent terminal deletions (no extra subtelomere signal) for chromosomes 4p, 10q, 13q, 22q, 22q, 22q, Yq, and Yq. Absence of a non-homologous subtelomere signal on the deleted chromosome is likely to correlate with a de novo deletion, but translocations of a reciprocal site distal to the subtelomere probe site would have a significant recurrence risk and can only be detected by parental studies. Two of the 11 patients inherited unbalanced derivative translocation chromosomes, der(18)t(4;18) and der(10)t(8;10) (2/11 = 18.1%). One of the 11 cases, referred for subtelomere analysis only, showed two X and two Y chromosomes consistent with a follow-up 48,XXYY karyotype. In reviewing the G-banded chromosomes, 7 cases, the der(18), the der(10), the three 22qter's, and the two Yqter's could be described as truly "cryptic". Three cases had apparent deletions; 4p-, 13q- and 10q-. It is unclear at this time whether the deletion Yq's are significant. The 4.1% of cases with a subtelomere alteration is lower than the figures reported by Knight et al., and Slavotinek et al., (7.5% J Med Genet 1999) and closer to the 4.9% figure reported by Lamb et al. (Abst ACMG 2001). Removing those cases which we describe as visibly identifiable, we calculate a lower percentage of cases with a "cryptic" subtelomeric alteration (6/270 = 2.2%), although a focused analysis could alter what is considered visible.

**Cryptic terminal deletions: Are they precursors to chromosome rearrangements?** *K.S. Reddy, X. Yang.* Dept Cytogenetics, Quest Diagnostics, Inc, San Juan Capist, CA.

For the first time we report two cases with chromosome rearrangements probably subsequent to a submicroscopic terminal deletion. The first case had a 46,XX,der(1)t(1;9)(p36.3;p13).ish der(1)(wcp9+)[88]/46,XX[12] karyotype. Mother has normal chromosomes the father is not available. A FISH scrutiny using wcp9, 1ptel and pan telomeric probes found a subtelomeric 1ptel deletion on the der(1) and on a chromosome 1 in the apparently normal cell line. The telomere (TTAGGG)<sub>n</sub>, however, was present on both copies of chromosome 1 in the apparently normal and abnormal cell lines. The unexpected subtle terminal deletion was detected by FISH when the abnormality was teased apart to draw a genotype to phenotype correlation. The second case had a de novo 46,X,der(X)t(X;22)(p23;q11.2),inv dup(22)(q11.2)[85]/45,X,der(X)t(X;22)(p23;q11.2),-inv dup(22)[15] karyotype. The complex rearrangement was further assessed using FISH paint probes for chromosomes X and 22, TUPLE-1/ARSA, BCR/ABL, Xptel, pan telomere, STS and KAL probes. The unexpected finding was a terminal deletion of Xpter distal to the KAL gene. The mechanism involved in the generation of these two rearrangements is probably initiated by a pre-zygotic terminal deletion. Case 1: a deletion of chromosome 1p36.3 in the zygote was followed by a somatic telomere healing of one deleted chromatid and a recombination between the other deleted 1p36 and 9p chromatids. The only two lines that survived were the original subtelomeric deletion of chromosome 1 cell line and the der(1) cell line. Case 2: Possibly during meiosis, a terminal deletion of Xp23 was stabilized by a recombination between X and chromosome 22. The inv dup(22) was formed by an U-type exchange. A second cell line was derived by a post-zygotic loss of the inv dup(22). These two cases suggest that terminal deletions are prone to recombination with another chromosome/chromatid if they do not heal with a telomere (TTAGGG)<sub>n</sub> cap.

**A novel method to detect parental origin of rea(21q21q).** *B.B. Wang<sup>1,2</sup>, W.L. CHEN<sup>1</sup>, H.C. LU<sup>1</sup>, L.J. HSIEH<sup>1</sup>, M.H. LEE<sup>1</sup>, C.J. GUO<sup>1</sup>.* 1) Department of Medical Research and Education, Changhua Christian Hosp, Changhua, Taiwan; 2) Department of Laboratory Medicine, Changhua Christian Hosp, Changhua, Taiwan.

Historically, homologous rearrangements of 21q have been termed Robertsonian translocations. This implies a translocation between two different chromosomes. Traditional cytogenetic methods can not distinguish between a true Robertsonian translocation and an isochromosome involving 21q. However, using molecular polymorphisms and Southern blotting hybridization, the majority of rea(21q21q) have been recently shown to be isochromosomes, of which parental origins were equally divided between two parents (Shaffer et al, 1993). In this study, we developed a novel non-radioisotope labeling method and successfully identified the parental origin of rea(21q21q) in a patient with Down syndrome. We used a panel of 8 highly polymorphic microsatellite markers for chromosome 21 and 8 internally labeled primer sets for non-isotopic amplification and detection. Unlabeled primer sets of DNA markers were extended briefly in the presence of infrared dye (IRD700) -labeled dATP prior to PCR amplification. Without further purification, the amplified products were applied directly to the denaturing sequencing gel, then detected and analyzed with a Li-Cor Model 4200L automatic DNA sequencer. The results revealed that two out of the 8 markers were informative and parental origin of the rea(21q21q) chromosome was maternal. This non-isotopic detection system has proven to be effective and straightforward in determining parental origin of homologous arrangements of 21q.



**Serendipitous detection of low-level somatic mosaicism in the mother of a child with deletion 5p14.2: limitation of "limited studies" in relatives of cytogenetically abnormal individuals.** *P. Van Tuinen<sup>1</sup>, J. Grignon<sup>2</sup>, P. Miller<sup>2</sup>, P. Schalk<sup>2</sup>, M. Emery<sup>2</sup>, C. Brooks<sup>2</sup>, L. Weik<sup>3</sup>.* 1) Medical College of Wisconsin, Milwaukee; 2) Dynacare Laboratories, Milwaukee; 3) Children's Hospital of Wisconsin, Milwaukee.

A child with dysmorphic features and hyperactivity was found to have a deletion 5p14.2. Although this is among the larger deletions seen in Cri-du-Chat syndrome, the child's features were considered surprisingly mild compared to most children with this syndrome. As in our standard work-ups in all such cases of structural rearrangement, a recommendation was made to consider peripheral blood studies of the parents to rule them out as carriers of a balanced rearrangement that may predispose them to bear further abnormal offspring. Several months later the mother was again pregnant, and both parents elected to be tested. For relatives of a bearer of a cytogenetic abnormality, our analysis, as in many cytogenetics labs, is a "limited study" consisting of five metaphase cells, since it is focused on the presence or absence of a known abnormality. The father's study was normal in five cells. In the mother five metaphases chosen randomly for the study were normal. However, the technologist evaluating G-banding had noted deletion 5p, initially considering it a spurious finding in light of the apparently "normal" result. Further searching quickly revealed a second deletion 5p. Nevertheless, in an extended study of 90 metaphases, only 4 were found to bear the deletion. The detection of what proved to be a low level of mosaicism was considered to be opportune and serendipitous. Obviously the probability of detection would have been nearer a certainty in a full standard study of 20 metaphases. While limited studies are valid for working horizontally or downward in a pedigree, our use of limited studies when working upward in a pedigree was brought into serious question with this case.

**Genetic variation in meiotic recombination in the male mouse.** *K.E. Koehler, J.P. Cherry, A. Lynn, J.H. Nadeau, T.J. Hassold.* Department of Genetics, Case Western Reserve Univ, Cleveland, OH.

Studies from model organisms such as *Drosophila* have shown that the frequency of meiotic exchange varies among different genetic backgrounds. Such effects are obviously more difficult to demonstrate in humans, and in mammalian model organisms (e.g., the mouse), conventional linkage analyses cannot be performed in inbred strains. However, recently developed immunofluorescence methodology, involving direct analysis of recombination-associated proteins in pachytene stage meiocytes, now makes it possible to circumvent these obstacles. Specifically, substantial evidence suggests that the mismatch repair protein MLH1 is a component of the late recombination nodule, and the number and distribution of MLH1 foci precisely mimic meiotic exchange patterns. Here we employ this technology to assess recombination patterns in male mice.

We find that among four different inbred strains - SPRET/Ei, C57BL/6, A/J, and CAST/Ei - the mean numbers of autosomal MLH1 foci per cell are significantly different. These means range from a high of 24.9 in SPRET/Ei to a low of 21.5 in CAST/Ei; therefore, in CAST/Ei males, most homologous chromosomes or bivalents are held together by only the single "obligate" crossover rather than multiple exchanges. From our observations of over 10,000 autosomal synaptonemal complexes, we conclude that non-exchange bivalents only rarely arise in the male mouse. We also present evidence suggesting that with advanced age the meiotic process in male mice may begin to break down, resulting in decreasing interference and more variability.

Although genetic background effects have long been suspected, this study provides the first direct, systematic demonstration of genetic variation in meiotic recombination between inbred strains of the laboratory mouse.

**Molecular cytogenetic studies of synapsis and recombination in infertile males.** *S. Mehra*<sup>1,2</sup>, *S. Schwartz*<sup>1,2</sup>, *A. Seftel*<sup>1,2</sup>, *L. Judis*<sup>1</sup>, *E. Chan*<sup>1</sup>, *T. Hassold*<sup>1,2</sup>. 1) Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Univ Hospitals of Cleveland, Cleveland OH.

Application of immunofluorescence methodology to mammalian pachytene preparations now makes it possible to visualize expression of meiotic recombination pathway proteins. This methodology also has potential clinical significance: it seems likely that abnormalities in the recombination machinery are a contributor to infertility. To test this, we have begun to examine pachytene preparations from infertile males. Meiotic preparations from men undergoing testicular biopsies for azoospermia/oligospermia were processed using CREST antiserum to identify kinetochores, and antibodies against SCP3 to identify synaptonemal complexes (SCs) and MLH1 to identify late recombination nodules, the sites of crossing-over. To date, we have analyzed 11 individuals with obstructive azoospermia but with normal spermatogenesis on histology ("Controls") and 5 individuals with non-obstructive azoospermia/oligospermia ("Cases"); individuals were pre-screened to rule out constitutional abnormalities or Yq microdeletions. For most individuals, 50 or more cells were analyzed. There were no obvious abnormalities in pairing/synapsis in any of the 11 controls. However, in 1 of the 5 cases, the normal complement of 23 SCs was replaced by 46 unpaired axial elements, resulting in complete meiotic arrest. This may reflect defects in proteins such as SCP1 that comprise the central portion of the SC; tests are now underway to test this. There were no obvious defects in the remaining 4 cases. However, the mean number of meiotic exchanges per cell, determined by counting MLH1 foci, was slightly but significantly lower than controls. Possibly, disturbances in recombination are a feature of any perturbation of spermatogenesis. Our results suggest that this approach will be useful in characterizing specific meiotic "phenotypes" in infertile males, thus providing an entree to mutation detection analyses. Further, the observation of reduced recombination in some infertile males may provide a reason for the increased rate of aneuploidy frequently seen in sperm of these individuals.

**High resolution mapping of centromeric satellite DNA families** . Y.C. Li<sup>1,3</sup>, C. Lee<sup>2</sup>, T.H. Hseu<sup>3</sup>, S.Y. Li<sup>1</sup>, C.C. Lin<sup>1,4</sup>. 1) Dept Life Sci, Chung Shan Medical/Dental Col, Taichung, Taiwan; 2) Dept. Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, USA; 3) Department of Life Sciences, National Tsing Hwa University, Hsinchu, Taiwan; 4) Dept Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada.

Centromere is an important domain responsible for proper segregation and transmission of chromosome during mitosis and meiosis. Higher eukaryotic centromeres including those of human comprise of mainly megabases of tandem repeated satellite DNA families. Some of these sequences have been implicated in centromere structure and/or function, but their genomic organizations at large remain uncharacterized due to the repetitive nature and complexity. We have applied multiple color FISH and fiber FISH to analyze the organization of three newly isolated cervid satellite DNA families (cervid sat.I, II, and III) in the centromeric/pericentromeric regions of Chinese water deer (*H. inermis*) chromosomes. Satellite II and satellite I were found to be located at the primary constriction and pericentric region respectively in most of the chromosomes of the complement. Satellite III usually was observed in the acrocentric chromosomes and was located toward to the short arm. The order of these three satellite DNA families appeared as pter - Sat.III - Satellite II - satellite I - qter. These satellite DNAs formed contiguous arrays with satellite I and satellite II juxtaposed to each other. The length of each satellite DNA array in a given centromeric region was also estimated based on the extended chromatin fiber preparation of ~10 kb/mm.

**Toward an analysis of the chromatin structure of the X-inactivation center.** *N. MISE, P. AVNER.* Mouse Molecular Genetics Unit, Institut Pasteur, Paris, FRANCE.

X chromosome inactivation is a chromosome wide phenomenon controlled by a cis-acting element called the *Xic* (X-inactivation center). Recent analysis has suggested that dynamic structural changes in chromatin structure contribute to the action of the *Xic*. To analyze the protein components and the structural change of the *Xic* chromatin during X-inactivation, we are establishing a native *Xic* chromatin isolation protocol from mouse embryonic stem (ES) cells carrying an *Xic* transgene.

As starting material for chromatin isolation, we have established a male BAC based transgenic ES cell line having a functional multicopy arrayed *Xic* transgene. The BAC clone used for the transgenesis covers the reported *Xic* region including the *Xist/Tsix* locus. Because the BAC contains a loxP site in the vector region, tandem arrayed transgenes can be excised in circular form by Cre recombinase. The BAC has been modified to contain a Neo resistant gene cassette allowing stable maintenance in ES cells and was tagged with a lac operator tandem repeat, so that the chromatin of the *Xic* transgene could be purified using a lac repressor protein affinity column taking advantage of the strong and specific binding between the lac operator and lac repressor protein. We have also constructed Cre expression vectors for inducible expression system in ES cells. The pANMerCreMer construct encodes a fusion protein MerCreMer which consists of Cre linked to two mutant mouse estrogen receptor ligand binding domains (Mer). Whilst this fusion protein has no recombinase activity in normal culture condition, the Cre is activated in the presence of the estrogen analogue OH-TAM (hydroxy-tamoxifen). Using this construct, we can synchronously excise *Xic* chromatin in vivo. The excised *Xic* chromatin is extracted from nuclei and is purified from the nuclear extract. Because the nuclear extract will contain many contaminated proteins unrelated to the *Xic* chromatin, a lac repressor protein affinity column has been used for purifying the lac operator tagged *Xic* chromatin. A progress report on our experimental approach will be presented.

**Conservation of FRA3B and FRAXB in the great apes.** *A.M. Loeb, M.F. Arlt, T.A. Morrish, T.W. Glover.*

Department of Human Genetics, University of Michigan, Ann Arbor, MI.

Common fragile sites are loci characterized by gaps or breaks on metaphase chromosomes from cells grown under conditions that inhibit DNA replication. Such conditions have been observed to induce fragile sites in several mammalian species, including dog, cat, pig, horse, cow, Indian mole rat, deer mouse and laboratory mouse. Some fragile sites in these species appear to be at sites homologous to human loci containing common fragile sites, but few direct tests of fragile site homology have been performed. It has been proposed that common fragile sites could play a role in chromosome evolution, as these sites are characteristically unstable after induction, showing increased rates of translocation, deletion and sister chromatid exchange, and are preferred sites for plasmid integration. FRA3B and FRAXB are two extensively studied fragile sites that each span hundreds of kilobases. FRA3B lies within the *FHIT* gene at 3p14.2, and it is known that *FHIT*/FRA3B is frequently deleted and translocated in tumors. FRAXB spans across the *STS* and *GSI* genes at Xp22.3 and is also unstable in some tumors. We have studied expression of FRA3B and FRAXB in several primate cell lines by FISH analysis with YACs known to span these fragile sites in humans. The YAC probes for both FRA3B and FRAXB were observed to cross their respective homologous fragile sites in the three great apes studied, chimpanzee, orangutan and bonobo, as well as in baboon, an old world monkey. Thus, both FRA3B and FRAXB appear to be conserved in these primates. In gibbon, a lesser ape, YAC probes for FRAXB were observed to cross a fragile site; however, probes for FRA3B were not observed to cross a fragile site. RT-PCR analysis indicates that the *FHIT* ortholog is expressed in this gibbon cell line, indicating that the gene is not extensively rearranged. These studies thus show that fragile sites are highly conserved in great apes but suggest that FRA3B may diverge in gibbons or the lesser apes. Furthermore, comparison of the FRA3B region between gibbon and species expressing this fragile site may allow insight into the mechanism of instability.

**A large intragenomic duplication homologous to human chromosome 3p21-24 during hominoid evolution.** *E. Zend-Ajusch, F. Gruetzner, U. Haensel, R. Sudbrack, H.H. Ropers, T. Haaf.* Max Planck Institute for Molecular Genetics, Berlin, Germany.

By hybridizing >80 YACs which are evenly spaced on human (HSA) chromosome 3 to chromosomes of orangutan (*Pongo pygmaeus*, PPY), gibbon (*Hylobates syndactylus*, HSY), Old World monkey (*Presbytis cristata*, PCR), and New World monkey (*Callithrix geoffreyi*, CGE), we have reconstructed the hypothetical ancestral chromosome, which is most similar to PCR 1. Two pericentric inversions each are required to derive HSA 3 and Borneo PPY 2 from this ancestral chromosome. An additional pericentric inversion separates the Sumatra PPY 2 from Borneo PPY 2. HSA 3 and Borneo PPY 2 share four large segments of conserved chromosomal synteny. Two smaller (1-3 Mb) segments have changed their chromosomal position by transposition. Evidently, chromosomal phylogeny is much more complex than suggested by classical chromosome banding and painting studies. We have identified YACs/PACs containing evolutionary breakpoints in HSA 3p25, 3p24.3, 3p13, and 3q21. These regions are also frequently involved in pathological chromosome rearrangements. Two of the evolutionary breakpoints are very close to olfactory receptor genes, which may promote illegitimate recombination between distant regions. Segments homologous to HSA 3 are found on two New World monkey chromosomes, CGE 17 and CGE 19, and on four gibbon chromosomes, HSY 1, HSY 10, HSY 17, and HSY 21. The series of derived chromosome rearrangements (translocations) in gibbon led to a duplication of a large (approximately 20 Mb) segment on HSY 1 and HSY 10. The duplicated segment is homologous to HSA 3p21-24 and contains approximately 100 genes. Smaller segmental duplications comprising only sequences of one YAC from HSA 3p21-24 are found on HSA 1q, PPY 1q and PCR 6q. There is no detectable duplication in the New World monkey. Our results suggest that large intragenomic duplications have occurred during recent primate evolution. Unique segmental duplication of a large number of genes and subsequent divergent mutations may be an important mechanism for acquiring genetic diversity and, thus, drive species evolution.

**A study on the relationship between chromosome polymorphism and reproduction abnormality.** *D.S. Tang<sup>1</sup>, Y.B. Tian<sup>1</sup>, L.H. He<sup>1</sup>, S.Q. Zeng<sup>1</sup>, X.J. Li<sup>2</sup>, W.P. Liu<sup>2</sup>, G. Yang<sup>2</sup>, Z.H. Chen<sup>3</sup>, X.L. Guo<sup>3</sup>.* 1) Dept Animal Sci, Foshan Univ, Foshan, Guangdong People's Republic of China; 2) The First Hospital of Foshan Foshan, Guangdong People's Republic of China; 3) The Women and Children Hospital of Foshan Foshan, Guangdong People's Republic of China.

The relationship between chromosome polymorphism and reproduction abnormality was investigated by comparing chromosome polymorphism group to normal karyotype group. Methods: Blood samples of health examination people with normal phenotype were collected, cell cultured and G-band analyzed. Karyotypes of some samples were analyzed by high-resolution banding. 158 people with chromosome polymorphism were regarded as test group. Control group of 206 people was sampled from other people with normal karyotype. The rates of reproduction abnormality of two groups were compared. The reproduction abnormality included abortion, infertile and baby abnormality, etc. Results: There was 46.8% (74/158) people with reproduction abnormality in polymorphism group and 23.3% (48/206) people with reproduction abnormality in control group. 29.1% (46/158) people had spontaneous abortion in polymorphism group and 15.5% (32/206) people had spontaneous abortion in control group. There was a significant difference between two groups ( $P < 0.01$ ). Half of abortion people had abortion in 2 month pregnancy in polymorphism group, compared to 25% in control group. Discussion: Studies of other groups didn't show the relationship between chromosome polymorphism and reproduction abnormality or cancer. But many doctors found some people with chromosome polymorphism had reproduction abnormality. Having reviewed some studies in other papers, we found these studies compared the rate of polymorphism in abnormal phenotype group with normal phenotype group. The two rates were too low to be compared. We compared the rate of abnormal phenotype in polymorphism group with the rate in normal karyotype group. The results showed that there was a relationship between chromosome polymorphism and reproduction abnormality. The chromosome polymorphism maybe resulted in reproduction abnormality.



**Molecular and cytogenetic characterization of translocations involving 22q11.** *E. Spiteri<sup>1</sup>, R. Goldberg<sup>1</sup>, L. Edelmann<sup>2</sup>, S.W. Gogineni<sup>1</sup>, V.R. Pulijaal<sup>1</sup>, A. Shanske<sup>1</sup>, K. Wakut<sup>3</sup>, C.D. Kashork<sup>3</sup>, L.G. Shaffer<sup>3</sup>, B.E. Morrow<sup>1</sup>.* 1) Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY; 2) Mount Sinai School of Medicine, NYC, NY; 3) Baylor College of Medicine, Houston, TX.

Chromosome 22q11 is susceptible to recurrent constitutional rearrangements associated with congenital anomaly disorders. Velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS) is associated with a partial monosomy of 22q11. Offspring of carriers of the constitutional t(11;22) translocation are susceptible to derivative (22) syndrome. These patients carry a supernumerary der(22) chromosome resulting in a trisomy of proximal 22q and distal 11q. Breakpoints for these disorders are clustered in three regions of 22q11 coinciding with low copy repeats (LCR22s) 200 kb in size. Homologous recombination events between LCR22s mediate the 1.5 and 3 Mb VCFS/DGS deletion. The t(11;22) translocation is mediated by AT-rich palindromes in one of the LCR22s (LCR22-3a). Non-recurrent translocations also occur in the 22q11 region associated with congenital anomalies. We have ascertained two such patients, one has a t(1;22) and the second has a t(18;22) whose translocation breakpoints occurred in LCR22-3a, suggesting a common mechanism. To investigate this possibility or to identify other hot spots in the 22q11 region, we are defining the translocation breakpoints in 13 other unrelated translocations by STS and FISH mapping approaches. Identifying the breakpoints will lead to a better understanding of the mechanism of translocation formation and define hotspots for chromosomal breakage leading to rearrangements. In addition, LCR22-3a has not been completely cloned, perhaps due to the presence of the AT-rich palindrome. We are currently mapping this region in non-human primates to determine the evolutionary origin of this unstable region in the genome.

**CGH IN THE EVALUATION OF THE PLACENTA IN ABNORMAL PREGNANCIES.** A. Amiel<sup>1,2</sup>, N. Bouaron<sup>2</sup>, M. Fejgin<sup>1,2</sup>, E. Gaber<sup>1</sup>, R. Sharony<sup>1,2</sup>. 1) Genetic Institute, Meir General Hosp, Kfar-Saba; 2) Sackler School of Medicine, Tel-Aviv University, Israel.

Comparative genomic hybridization (CGH) is a FISH related technique used to assess global chromosomal aberrations in variety of human tumors. Recently CGH has been utilized for cytogenetic analysis of fresh, frozen and paraffin embedded feto-placental tissues. We applied CGH technique to samples taken from various sites of placentas originating from abnormal pregnancies (IUGRs, fetal malformations, toxemia, hydrocephalus, ante-partum fetal death and undetectable MSAFP) and controls. Among the four IUGR cases, one placenta was with trisomy 13 in one of the sites and one placenta with 47,XXY in all sites and trisomy 8 in one site. In the case with MSAFP=0, monosomy 16 was detected in two sites. In the control cases (7 normal and 5 with aneuploidy) CGH results concurred with the known karyotype. Our result demonstrate the usefulness of CGH technique in the genetic evaluation of the placenta of problematic pregnancies even if its morphology is normal.

**No accelerated telomere shortening on the Y chromosome with age.** *L. Chavez<sup>1</sup>, E. Rajcan-Separovic<sup>2</sup>, P. Lansdorp<sup>1</sup>*. 1) Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, Canada; 2) Cytogenetics Laboratory, BC Children's Hospital, Vancouver, Canada.

It has recently been shown that telomere shortening with age does not affect all the chromosomes equally. Surralles et al (*Am. J. Hum. Genet*, 1999, 65:1617-1622) measured the length of the inactive X chromosome telomeres in young and older females and showed that telomere repeats are lost from the inactive X chromosome at an accelerated rate. These authors proposed that accelerated telomere shortening renders the inactive chromosome unstable relatively early in life and that this may have a role in the observed phenomenon of chromosome X aneuploidy in the peripheral blood of elderly females.

A similar, age-related loss of the Y chromosome is observed in hematopoietic cells in the bone marrow of elderly males. This finding frequently represents an interpretational dilemma since it is hard to distinguish between the age-related loss of the Y chromosome and a neoplastic change. We have used quantitative FISH to evaluate the rate of telomere loss at the Y chromosome relative to other chromosome ends in normal bone marrow cells from children and elderly males. We found no significant difference in the rate of telomere shortening in the Yq and Yp telomere compared to that in all autosomal telomeres (young males 2-17 yrs: average Yp= 8.78 TFU, average Yq=5.9 TFU, average autosome p=6.35 TFU; old males 54-71 yrs: average Yp= 6.65 TFU; average Yq=3.35 TFU; average autosome p= 4.15 TFU). These results suggest that other mechanisms than accelerated telomere shortening are responsible for the loss of the Y chromosomes with age. Interestingly, significantly longer Yp telomeres were found in cells from 6/8 individuals from both age groups, indicating that Yp telomeres are typically among the longer telomeres in the genome.

**A rec(7)dup(7q)inv(7)(p22q23.2) derived from an inverted maternal chromosome 7, inv(7)(p22q31.2) in a new born.** A. Babu, T. Shlovsky, S. Kleyman, V. Mizhiritskaya, M.J. Macera. Dept Mol Medicine & Genetics, Wyckoff Heights Med Ctr, Brooklyn, NY.

Chromosomal analysis of peripheral lymphocytes from a new born revealed an abnormal 46,XY,rec(7)dup(7q)inv(7)(p22q31.2) karyotype. Follow-up analysis of maternal blood revealed an abnormal 46,XX,inv(7)(p22q31.2) karyotype. The recombinant chromosome was further characterized using directly labeled probes, Elastin located at 7q11.23, and D7S486 and D7S522 located at 7q31.3 on both proband and maternal chromosomes. In the mother, the inv(7) had elastin signal located in the proximal short arm, opposite to the centromere from D7S486,D7S522 at 7q21.3, while a normal pattern was seen on the 7 homologue. In the proband, the recombinant 7 had two sets of signals corresponding to D7S486,D7S522, one on each side of the centromere with only a single signal for the elastin locus. This confirms the breakpoint in the inv(7) at q31.2 and establishes the presence of two copies of band 7q(7q31.3 -> qter) in the proband. The entire rec(7) is positively labeled by wcp 7. The final karyotype of the proband is 46,XY,rec(7)dup(7q)inv(7)(p22q31.2)mat .ish rec(7)(wcp+, Elastin+, D7S486, D7S522+,+). The recombinant chromosome 7 originated during maternal meiosis I, owing to a cross over between the inv(7) and the normal 7 homologue within the inverted region. The product of the crossing over gave rise to the rec(7)dup(7q)inv(7)(p22q31.2), which has a very small monosomy for the 7p terminal region (7p22->pter) and a trisomy for the 7q terminal region (7q31.2->qter). It is interesting that the location of the breakpoint in this case, is at 7q31.2. There is strong evidence showing genes that contribute to both autism and specific developmental disorders of speech and language (SDDSL) are located on chromosome 7 band 3q. [Vincent et al 2000, Lai et al 2000, Warburton et al 2000, Ashley Koch et al 1999, Folstein and Maknoski 2000]. Due to the duplication of the critical region 7q31.2, the proband is being followed, and additional molecular characterization of the recombinant chromosome is underway.

**Microdeletions and cryptic translocations detected by FISH with multiple subtelomeric probes and spectral karyotyping in idiopathic mental retardation.** *Y.S. Fan<sup>1</sup>, Y. Zhang<sup>1</sup>, M. Speevak<sup>2</sup>, S. Farrell<sup>2</sup>, J.H. Jung<sup>1</sup>, V.M. Siu<sup>1</sup>.*  
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Genomic alterations in the subtelomeric regions appear to be a frequent cause of mental retardation and developmental disabilities. However, the reported frequency of subtelomeric abnormalities in this population has been inconsistent. We have performed FISH testing using the Cytocell Multiprobe-T system on 149 patients selected based on the criteria: 1) mental retardation (IQ<70) or developmental delay with dysmorphic features; 2) a normal karyotype at the level of resolution of 450-500 bands; and 3) exclusion of other possible etiologies by a full genetic assessment and relevant tests. Abnormal findings were confirmed by spectral karyotyping (SKY) and Vysis probes, and family studies were carried out to determine inheritance. Clinically significant aberrations were detected in 6 proband patients (4%) including 1p36 deletion in two cases and unbalanced cryptic translocations in 4 cases, while deletion of the 2q subtelomeric region detected by probe D2S2986 appeared to be a common variant (6%). All the cryptic translocations were also identified by SKY. These results have demonstrated that both FISH with multiple subtelomeric probes and SKY are valuable clinical tests for establishing a definitive diagnosis for patients with idiopathic mental retardation / developmental disorders.

**Delineation of mosaic constitutional marker chromosomes using CGH, m-FISH and DNA analysis.** *B.L. Lomax<sup>1</sup>, O. Ludkovski<sup>2</sup>, S. Masui<sup>1</sup>, C. Brown<sup>3</sup>, DK. Kalousek<sup>1,2</sup>, E. Rajcan-Separovic<sup>1,2</sup>.* 1) Dept of Pathology, Women's and Children's Health Centre, Vancouver, BC; 2) Dept of Pathology, UBC, Vancouver BC; 3) Dept of Medical Genetics, UBC, Vancouver BC.

Supernumerary marker chromosomes are a heterogeneous group and precise characterization is necessary to establish their potential clinical significance. We have studied two patients who presented with behavioral problems but no dysmorphic features (Case 1) and dysmorphic features and learning problems (Case 2). In both cases, conventional cytogenetic analysis demonstrated the presence of a variable number (1-3) of small marker chromosomes in <50% of cells. C-banding suggested an euchromatic (C-band negative) component in both cases.

Multicolor FISH analysis (m-FISH) showed that in Case 1 both markers originate from chromosome 8, while in Case 2, 1 out of 3 markers showed chromosome X specific painting. The remaining 2 markers in Case 2 were not painted by m-FISH. CGH showed a slight gain at chromosome 8p11.1-q13 in case 1, and at the X chromosome centromere in case 2. Conventional FISH analysis confirmed that both markers in Case 1 hybridize with chromosome 8 paint, while only one hybridized with chromosome 8 a satellite. In Case 2, only one of the markers hybridized with the X chromosome a satellite and both the Xp and Xq arm-specific paints. Co-hybridization of chromosome X a satellite and the arm-specific probes showed co-localization of the two colors on one marker chromosome reflecting the presence of centromeric repeats in the microdissected arm-specific probes and precluding further cytogenetic characterization. Pericentromeric DNA markers will be used in an attempt to establish presence of euchromatin in this marker.

We can conclude that although the chromosomal origin can be suggested or confirmed by CGH and m-FISH, determining the euchromatic component of small marker chromosomes will remain a challenge due to the unavailability of FISH probes solely for euchromatic regions. The elucidation of small markers not painted by m-FISH will be facilitated in the future with the development of centromere specific m-FISH (cenM-FISH).

**The Association of Subtelomeric Deletions and Severe Mental Retardation.** *J.H. Hersh<sup>1</sup>, M.J Barch<sup>1</sup>, F.F. Yen<sup>1</sup>, J.A. Axelrod<sup>1</sup>, G. Gowans<sup>1</sup>, L. Sears<sup>1</sup>, A. Parsian<sup>2</sup>.* 1) Weisskopf Center for the Evaluation of Children/Dept Pediatrics, Univ Louisville, Louisville, KY; 2) School of Dentistry, Univ of Louisville, Louisville, KY.

Mental retardation occurs in about 3 percent of the general population. Although the likelihood of determining its etiology increases with the severity of the cognitive deficits, the cause remains unknown in up to 40 percent of cases with moderate to severe mental retardation. Recently, it has been demonstrated that cytogenetically invisible chromosomal rearrangements involving the subtelomeric regions represent an important cause of mental retardation. Knight et al reported the presence of subtle chromosomal abnormalities in 7.4 percent of children with moderate to severe unexplained mental retardation identified by a multiprobe telomere FISH protocol. Using the same approach with a ToTel Vysion probe panel (Vysis cat.33-270000), we have completed studies on 44 children with severe developmental delay/mental retardation of undetermined etiology. In 3 (6.8 percent) cases, a subtelomeric deletion was identified involving 1p, 1q and 2q. In 2 (4.5 percent) patients, parental telomere studies were normal, and in the third, results are pending. Our findings are similar to those previously reported, and support the notion that subtelomeric rearrangements represent an important cause of unexplained severe mental retardation. Therefore, a search for subtelomeric deletions using a multiprobe FISH protocol should be included in the diagnostic evaluation, when the etiology of the mental retardation is not readily apparent in this patient population.

**Sequential M-FISH and Rx-FISH in delineating the origin of ambiguous chromosome regions / fragments. C.**

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Elucidating the origin of de novo marker chromosomes or ambiguous chromosome regions may be clinically significant and provides valuable data on phenotypic and aneuploidy relationships. However, in the clinical cytogenetics laboratory, determining the origin of these chromosome regions can be difficult and laborious when relying on conventional banding techniques, individual chromosome paints and/or locus-specific probes. Multicolor karyotyping systems are ideal technologies for rapidly identifying the composition of these markers / derivative chromosomes. Multiplex (M-) FISH and spectral karyotyping (SKY) in humans use a probe set containing differentially-labeled, chromosome-specific human paints to simultaneously identify each of the 24 non-homologous chromosomes with a unique spectral profile (color). Rx-FISH uses differentially-labeled chromosome-specific gibbon chromosomes which when hybridized to human chromosomes, produces a specific banding pattern on human chromosomes. Here, we illustrate the value of sequential M-FISH and Rx-FISH in a clinical case: Chromosome studies from a chorionic villus sample revealed a fetal karyotype of 46,XY,add(9)(p24) by GTG-banding. Chromosome analysis by GTG-banding on peripheral blood lymphocytes was normal for both parents. The pregnancy was continued and led to the birth of a full term, dysmorphic male infant. Subsequent peripheral blood karyotyping by GTG-banding confirmed the previous CVS chromosome results. M-FISH on the peripheral blood chromosome sample revealed that the additional chromosome material came from chromosome 2. Rx-FISH more specifically identified the additional material as originating from the distal short arm of chromosome 2. Together with a review of the G-banded chromosomes, the revised karyotype was 46,XY,add(9)(p24)*de novo*.ish der(9)t(2;9)(p21;p24)(wcp2±,wcp9±).



**Partial Trisomies and Deletions Identified by FISH, M-FISH, and Subtelomeric Probes.** *C.B. Lozzio, T. Ryan, E. Bamberger, E. Ralston, E. Holland, W. Carter, C. Worthington, K. Harmon.* University of Tennessee Department of Medical Genetics, Knoxville, TN.

Examples of chromosome rearrangements identified by molecular cytogenetic techniques are reported. One case of partial trisomy 14q and monosomy 18p was found in a 6 years old Hispanic male with severe growth and mental retardation, mild dysmorphic features, and history of duodenal atresia. The initial karyotype showed a "de novo" add(18)(p11.2) and the FISH studies with Vysis ToTelVysion Probe Panel for subtelomeric probes demonstrated a der(18)t(18;14)(q32.1;p11.2). Monosomy 8p and partial trisomy 13q with karyotype 46,XY,der(8)t(9;13)(p23;q22) was also identified using the Vysis subtelomeric probes in a 14 month old white male with craniofacial asymmetry, mild dysmorphic features and severe developmental delay. Telomeric deletions 2q37 have been frequently found as polymorphic familial variants using the Cytocell Multiprobe-T. We observed a true telomeric deletion 2q37.3 visible at the 600 band level by cytogenetic analyses and confirmed with the Vysis 2q telomeric probe in a 2 years old white female with brachycephaly, dysmorphic features and developmental delay. Mosaicism for partial trisomy 5 due to the presence of a small marker chromosome was observed in two cases. The origin of the marker chromosome was determined by Multicolor FISH studies (M-FISH) using the Multicolor Spectra Vysion from Vysis and the Applied Imaging Cytovision version 4.1 for UNIX with software for M-FISH. This finding was confirmed with the whole chromosome 5 and centromeric probes. One case was a 2 years old black male with tall stature, relative microcephaly and severe developmental delay. The other was initially found in an amniocentesis and confirmed by cytogenetic analyses of peripheral blood after delivery. At 1 month of age this baby had normal development and did not show dysmorphic features. The first case had the extra marker in 54% of the cells and the second case showed the extra marker in 71% of the cells. Both cases appear to be trisomic for the same pericentric region of chromosome 5, however the clinical findings were severe in one case and very mild or absent in the other.

**The origin of abnormalities in recurrent aneuploidy.** *W.P. Robinson<sup>1</sup>, D.E. McFadden<sup>1,2</sup>, M.S. Stephenson<sup>3</sup>*. 1) Dept of Medical Genetics, Univ British Columbia, Vancouver, BC., BC, Canada; 2) Dept. of Pathology; 3) Dept. of ObGyn.

Recurrent miscarriage due to sporadic aneuploidies may simply be a consequence of the dramatic increase of trisomic conceptions with maternal age. However, it is possible that some couples are at increased risk as a result of gonadal mosaicism, factors affecting chromosome structure or segregation, or increased sperm abnormalities in the male partner. We report cytogenetic and molecular findings from 111 spontaneous abortions (SAs) from 49 couples ascertained as having two or more documented aneuploid or polyploid SAs. The mean number of SAs per couple was 4 and mean maternal age at time of loss was 37.8 (range 19-46) yrs. The distribution of abnormalities was compared to 210 chromosomally abnormal SAs diagnosed at the same center but without documented recurrent aneuploidy/polyploidy (although most were from women with other non-karyotyped SAs); Mean maternal age was 35.3 (range 21-47) yrs for the control group. Although recurrence of the same trisomy occurred in 6 families, this was not more often than expected by chance, indicating that gonadal mosaicism is rarely the explanation for recurrence. The origin of the trisomy was determined in 33 SAs from 22 of the couples in the study. A maternal meiotic origin was concluded for 28 cases, possible somatic (mat) for 2 cases, and a paternal meiotic origin in 3 cases. These results are consistent with data from sporadic trisomies and suggest a similar etiology (e.g. maternal ovarian aging). The distribution of abnormalities among the recurrent aneuploidy group was similar to that for the controls, although both groups showed more trisomy 15 as compared to previous reports. The proportion of trisomic SAs (as compared to monosomy or polyploidy) was significantly higher in the recurrent aneuploidy group overall (84% vs. 67%;  $P=.0005$ ) and, when stratified by age, was significant among mothers <32 (83% vs. 40%;  $P=.05$ ) and 32-35 years of age (75% vs. 50%;  $P=.05$ ), suggesting possible premature ovarian aging in younger women experiencing recurrent trisomy. Further studies are needed to confirm an increased predisposition to trisomy in these women and determine if some specific trisomies are over-represented.

**A familial 14Mb interstitial deletion of 21q11-q21.3 confirmed by FISH using subregional-specific DNA clones.**

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We previously reported a family with an interstitial deletion of 21q11-q21 including a mother and two children (ASHG meeting, 1999). Although the mother was phenotypically and mentally normal, and the first affected child had deafness and moderate mental retardation and the second affected child had only mild mental retardation. We report here the precise breakpoints of the deletion confirmed by FISH using subregional-specific DNA clones. Recently, the human draft genome sequence had been reported. The availability of the mapped and sequenced clones allows for accurate diagnosis of chromosomal abnormalities associated with various phenotypes. Forty-five different subregional-specific DNA clones (Nature, 2000) were used as FISH probes, and we proved that the proximal breakpoint of the deletion of 21q of three family members was located between P16C2 and P879G12 (~0.8 Mb region) and the distal breakpoint was located between RP11-45O19 and RP11-666H8 (~14.8 Mb region). Thus, this family has a 14Mb deletion within 21q11-q21.3. It is interesting that the phenotype of these family members are variable. As the mother is entirely normal, the dark band 21q21 perhaps is lower in gene content. However, it is possible that some recessive genes responsible for mental retardation or deafness exists within 21q11-q21.3. The children with the same deletion are affected with mental retardation and/or deafness and thus may represent an unmasking of a deleterious gene on the fathers chromosome.

**Molecular Cytogenetic delineation of a rearrangement involving the TSHR gene region at 14q31-32 resulting in a rare occurrence of Graves' Disease in early childhood.** *L.R. Shapiro<sup>1,2</sup>, T.F. Davies<sup>3</sup>, M.B. Damore<sup>2</sup>, D.L. St.*

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Graves' Disease is an autoimmune hyperthyroid disorder of adulthood resulting in thyrotoxicosis, exophthalmos, goiter and elevated T4 and T3, low TSH and elevated Thyroid Stimulating Antibody (TSHR-Ab). The occurrence of Graves' Disease during childhood is rare. The 14q31 region is the location of the genes for Graves' Disease, Thyroid stimulating hormone receptor (TSHR) and Congenital Hyperthyroidism without an autoimmune component.

A 4 year female old child with developmental delay was diagnosed with hyperthyroidism because of rapid heart rate and muscle weakness and found to have elevated T3 and T4 with low TSH and elevated TSHR-Ab, indicative of Graves' Disease. She was referred for genetic evaluation because of dysmorphic features and manneristic behavior and found to have exophthalmos consistent with Graves' Disease. Chromosome analysis revealed an apparently balanced de novo translocation [46, XX, t(4;14)(q23;q32)]. Comparative genomic hybridization was confirmatory. Because the products of the type 3 iodothyronine deiodinase (DI03) and TSHR genes at 14q31-32 are involved in thyroid function, FISH analysis using the D3 probe was done and revealed an intact FISH signal on the derivative 4q; therefore, the break point was proximal to 14q32 and the DI03 gene. In view of a likely 14q31 breakpoint and the clinical picture of Graves' Disease, a position effect on the TSHR gene or a disruption of the gene at the break point became a possibility. In order to determine if the TSHR gene is intact, a FISH probe was prepared from thyrotropin receptor cDNA, but the initial FISH attempt was unsuccessful. The TSHR FISH will be repeated, and depending on the results, a sequential FISH strategy and/or sequencing of the region will be considered.

**Deletion of the proteolipid protein gene (PLP) in a patient with Pelizaeus-Merzbacher disease.** *V.C. Thurston<sup>1</sup>, T.A. Gobbett<sup>1</sup>, L.R. Smith<sup>1</sup>, Q. Gao<sup>1</sup>, K. Inoue<sup>2</sup>, J.R. Lupski<sup>2</sup>, S.R. Dlouhy<sup>1</sup>, J.T.R. Clarke<sup>3</sup>, M.E. Hodes<sup>1</sup>, G.H. Vance<sup>1</sup>.*  
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Pelizaeus-Merzbacher disease (PMD) is an X-linked neurologic disorder characterized by dysmyelination in the central nervous system. Duplications of the proteolipid protein (PLP) gene located at Xq22 are estimated to cause 60% of cases of PMD, while point mutations in exons account for another 10-15%. We report here the second case of a deletion of the PLP gene observed in PMD. Peripheral blood was obtained from a patient referred for a diagnosis of PMD, presenting with developmental delay, spastic diplegia and an abnormal MRI. To determine whether he carried a duplication of the PLP gene, FISH studies were performed on interphase cells with a cosmid probe for the PLP gene and two control probes. Two hundred interphase cells were analyzed and all were deleted at the PLP locus. Cytogenetic analysis was normal. PCR analysis with primers for exon 4 of the PLP gene was negative for the PLP locus. FISH and haplotype analysis was also performed on the proband's mother and maternal grandparents. FISH analysis detected the deletion only in the patient's mother. Haplotypes of the proband and the grandfather were identical. This suggests the genomic recombination event that results in the PLP deletion may have occurred in the grandfather's meiosis. To determine the extent of the deletion in the proband, three overlapping BACs were hybridized to cells from the patient. One BAC that mapped to the telomeric side of the PLP gene was also deleted. Preliminary evidence indicates the size of the deletion is at least 73 kb. In the other report of a patient with a deletion of PLP, it was estimated that the deletion spanned at least 29 kb of genomic DNA. That patient had clinical features of PMD similar to patients with a duplication of PLP, and peripheral neuropathy. Further mapping of the deletion in our patient will be performed to determine more precisely the extent of the deletion and whether other genes in the area are also deleted.

**Molecular cytogenetic screening in CHARGE association.** *D. Sanlaville<sup>1</sup>, J.M. Lapierre<sup>1</sup>, D. Genevieve<sup>2</sup>, C. Ozilou<sup>1</sup>, M. Le Lorc'h<sup>1</sup>, S. Brisset<sup>1</sup>, P. Gosset<sup>1</sup>, C. Baumann<sup>3</sup>, J. Amiel<sup>2</sup>, S. Lyonnet<sup>2</sup>, C. Turleau<sup>1</sup>, M. Vekemans<sup>1</sup>, S.P. Romana<sup>1</sup>.*  
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CHARGE association is a non random occurrence of congenital malformations including coloboma, heart disease, choanal atresia, retarded growth and/or retarded development, genital hypoplasia, ear anomalies and/or deafness. The cause of this association remains unknown. Various genetic mechanisms were proposed, including, a subtle chromosome anomaly involving an unknown gene. To date individuals with CHARGE association phenotype and a visible cytogenetic anomaly have not provided any localization. To address this question, we decided to study 27 patients with CHARGE association and a normal standard karyotype with CGH and FISH using 41-subtelomeric probes. We found 2 chromosomal anomalies not yet described in CHARGE association: a der(9)t(9;13) derived from a parental translocation and a der(6)t(4;6) of unknown origin. Other observed anomalies were a dim(9p) observed using CGH in five patients and an unusual insertion of a small segment of 3p into 22q arm, inherited from the mother. We are currently trying to confirm the del(9p) using a YAC contig. Two regions (9p and 22q) are therefore potentially interesting to investigate by molecular tools in CHARGE association patients. Our results confirmed that chromosome anomalies might mimic CHARGE association and that more precise clinical criteria are needed. It is likely that the new diagnostic criteria of the CHARGE association proposed by Blake and al.(1998) could define a homogeneous-group of patients for which a single genetic cause might be identified. In conclusion a patient with CHARGE association must be tested with classical and molecular cytogenetic studies. Only a biological marker would allow the identification of a distinct clinical entity.

**Partial 9p monosomy in a child with a der(9)t(9;11)(p24;p15.4) identified by chromosome microdissection. D.**

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We report a 4 year old male with a partial monosomy for the regions 9p24 ® pter and a partial trisomy for the region 11p15.4 ® pter as a result of a de novo translocation; der(9)t(9;11)(p24;p15.4). The child was evaluated for severe developmental delay, failure to thrive and dysmorphic features including triangular facies, prominent forehead and wide nasal bridge. Other features included pectus excavatum and scoliosis. Chromosomal evaluation prior to his visit to the National Institutes of Health revealed a derivative 9 chromosome. High resolution G-banding failed to identify the unknown chromosomal material. Spectral karyotyping (SKY) was employed and was unsuccessful in identifying this chromosome material. Microdissection of the region spanning the presumed breakpoint identified the undetermined chromosomal material on 9p as 11p15.4 ® pter. This region was technically large enough for resolution with SKY; however, the involved combination of fluorors utilized for identifying chromosomes 9 and 11 were too similar for reliable detection. The critical region for the 9p-deletion syndrome has been mapped to 9p22-p23. While the proband shared features consistent with the 9p-deletion syndrome, his deletion is more terminal and excluded the critical region for the 9p-deletion syndrome. Chromosome microdissection may be used to delineate complex chromosomal rearrangements not identified by high resolution banding or spectral karyotyping.

**Prenatal interphase FISH analysis: Five year experience and unusual cases.** *G.D. Wenger, J. Labanowska, M. Millard, J.M. Gastier.* Children's Hospital Dept. of Laboratory Medicine and Ohio State University Dept. of Pathology, Columbus, OH.

FISH analysis for prenatal screening for aneuploidy involving chromosomes 13, 18, 21, X, and Y is becoming increasingly common. This testing, generally requested due to ultrasound abnormality, late gestational age, or abnormal maternal serum screening results, provides a rapid (24-48 hr) preliminary result to physicians and patients awaiting results of metaphase chromosome analysis. Currently, approximately 17% of amniocentesis samples in our lab are accompanied by a request for FISH analysis. Since participating in the clinical trial that led to FDA approval for the AneuVysion-TM probe panel in 1997, our experience with 477 cases indicates 100% concordance between FISH and conventional cytogenetics for the aneuploidies that the AneuVysion assay is designed to detect. 2.1% of tests (10 cases) were non-informative due to maternal cell contamination or insufficient volume/cells. Aneuploidies were detected in 14% of cases: 27 cases +21, 19 cases +18, 7 cases +13, 6 cases monosomy X, 6 cases triploidy, 2 cases with other sex chromosome abnormalities. Our experience includes a case with abnormal AneuVysion result (3 signals CEP18 probe) and an unusual karyotype: 46,XX,dic(15;18)(q24;q11.2),+18.ish dic(15;18)(D18Z1+)de novo. Our experience also includes 12 cases with no abnormalities detected by the AneuVysion screen but with an abnormal karyotype (3 derivative chromosomes, 3 inversions, 2 recombinant chromosomes, 2 deletions, 2 ring chromosomes) and 3 cases with abnormalities or rearrangements in addition to those detected by AneuVysion (dicentric or supernumerary chromosomes). 2.5% of cases thus had chromosome rearrangements that were not detected by the screening test, but these represent abnormalities not designed to be detected by the probe panel. Overall for this time period, 16.3% of AneuVysion tests performed had an abnormal result. This testing, accompanied by clear, thoughtful communication and counseling between laboratory, physician, and patient, represents an accurate, sensitive method for rapid detection of the most common aneuploidies in high risk pregnancies.



**FISH (Fluorescent in situ Hybridization) Confirming a Mosaic Trisomy 14 in a Newborn.** *J. Burns, M. Arsham, L. Adomaitis, J. Christian, R. Ligi, S. Kazimi.* Cytogenetics Lab, Danbury Hosp, Danbury, CT.

Newborn blood for chromosome analysis was received to rule out or confirm a suspected mosaicism for trisomy 14. Mosaics are individuals with two or more genetically different cell populations. Amniocentesis performed by two separate labs had found extra copies of chromosome 14 in some cells. Since these trisomic cells were found in more than one culture vessel, this mosaicism was considered to represent a true mosaicism, not an artifact of in vitro culturing. Physical findings at birth included organomegaly, short proximal limbs, a right auricular pit, and abnormal pinnae. The patient was transferred to a tertiary institution where she was later discharged home. Cytogenetics of peripheral blood revealed a karyotype of mos 47,XX,+14[5]/46,XX[95]. FISH studies confirmed this finding with 5/117 cells (1 metaphase and 4 interphase nuclei) having 3 signals for the telomere of chromosome 14. Placental tissue was also cultured and showed this mosaicism. The ISCN (1995) cytogenetic and FISH results are as follows: mos 47,XX,+14[49]/46,XX[74].ish 14(D14S308x3)[12] nuc ish 14(D14S308x3)[174]. This pregnancy most probably started as a trisomy 14 and rescue occurred when a daughter cell lost one copy of chromosome 14. As the pregnancy progressed, placental tissue continued to have the highest percentage of trisomic cells, followed by extraembryonic membranes (amnion and chorion), and finally the peripheral blood. Uniparental disomy (UPD) is a problem which can complicate this type of cell rescue from a trisomic to a diploid cell line. With UPD, both copies of the chromosome in question will have originated from one parent, while the lost chromosome will have been the only one contributed by the second parent. Since UPD for chromosome 14 has recently been shown to be associated with an abnormal phenotype, studies for this are being planned by the other institution, which is caring for this child. Hopefully the results will determine if the observed phenotypic anomalies seen are the result of mosaicism for trisomy 14, UPD, or a combination of both.

**47,XX,+8/47,XX,+13 karyotype in a newborn with multiple congenital anomalies.** *I.M. Amir<sup>1</sup>, B. Abolaimoun<sup>2</sup>, M. Al-Hathal<sup>2</sup>, K. Al-Hathlol<sup>2</sup>, W. Al-Eyaid<sup>2</sup>, S. Sultan<sup>3</sup>, M. Al-Balwi<sup>1</sup>, N. Al-Atwi<sup>1</sup>, K. Al-Tawi<sup>2</sup>.* 1) Dept Pathology, King Fahad National Guard Hosp, Riyadh, Saudi Arabia; 2) Dept Pediatrics, King Fahad National Guard Hosp, Riyadh, Saudi Arabia; 3) Dept Obstetrics and Gynecology, King Fahad National Guard Hosp, Riyadh, Saudi Arabia.

Cases with double autosomal aneuploidies are rare. We report the first case of its kind of a grossly abnormal baby girl with two separate cell lines, one with trisomy 8 and one with trisomy 13. Ultrasound examination of the fetus at 31 weeks gestation revealed hydrocephalus, Dandy-Walker malformation, absent corpus callosum, bilateral dilated renal pelvises, overlapping fingers and bilateral club feet with rocker-bottom. Chromosome analysis on a cordocentesis specimen showed trisomy 8 (65% of cells) and trisomy 13 (35% of cells). Examination of the baby after birth confirmed the antenatal findings and revealed other multiple organ anomalies that were features from trisomy 8 and trisomy 13 syndromes. The trisomy 8 and trisomy 13 cell lines were also demonstrated in skin fibroblasts. The baby was supported actively for two weeks and then was put on no code and died from heart failure.

**Case Report: De novo t(1;6) translocation with partial anodontia and speech delay.** *A.B. Decker, A. Asamoah, D.L. Van Dyke.* Department of Medical Genetics, Henry Ford Hospital, Detroit, MI.

We report on a 6.5-year-old girl with a balanced translocation between the short arms of chromosomes 1 and 6. She was referred for evaluation because of speech delay, and congenital absence of several deciduous and permanent teeth. She was reportedly very sensitive to noise (hyperacusis), had poor growth of the hair and nails, had decreased sweating and becomes red with high fever. When we saw her, she had microcephaly (head circumference at the second percentile; weight and height were at the 25th percentile), short palpebral fissures, epicanthal folds, sparse eyelashes, large ears, partial anodontia, short finger and toenails, and dry skin. She has mild developmental delay. Family history is significant for learning problems in two paternal uncles, one paternal aunt and several paternal cousins. Thyroid studies, calcium, phosphorus and alkaline phosphatase levels were within normal limits. Her karyotype was 46,XX,t(1;6)(p22;p22) and parental karyotypes were normal. This apparently balanced rearrangement might have resulted in either a submicroscopic loss of genetic material or loss of function of a gene that causes ectodermal dysplasia. There is no reported case of ectodermal dysplasia associated with this chromosome rearrangement. Further studies might help determine whether there is a gene locus for ectodermal dysplasia at or near either of the translocation breakpoints.

**Duplication 6q11-q15.** *L.M. Dellefave, C.B. Whitley, B. Hirsch.* Institute of Human Genetics and University of Minnesota Medical School, University of Minnesota, Minneapolis, MN.

We describe a patient with a small duplication within the long arm of one chromosome 6. The proband was referred to a Clinical Geneticist at Fairview University Medical Center at 5 yrs. of age for evaluation of developmental delay and a history of neurological problems including failure to thrive due to feeding difficulties, and at 3 yrs. of age, stumbling and an abnormal EEG. Evaluation at that time with the Bayley Mental scale showed functioning in the moderate range of mental retardation with an IQ of 46. Physical exam at 5 yrs. revealed OFC below the 5th percentile, height below 5th percentile, weight at 25th percentile, with developmental and speech delay. Craniofacial dysmorphism included hypertelorism, small mouth, and micrognathia. Gait abnormality, equinovarus, and flatfooted feet while standing were present. Neurological exam showed slightly increased ankle reflexes with normal deep tendon reflexes, strength, and tone. Chromosome analysis of peripheral blood lymphocytes revealed a duplication within the long arm of one chromosome 6 extending from band q11 to q15. Parental studies were performed and revealed mosaicism for the duplication of bands 6q11 to 6q15 present in one cell out of 50 in one parent.

Of the 26 reported cases of partial trisomy 6q, the majority involve duplications distal to band 6q15. Only one of the published cases had apparently the same 6q11 and 6q15 breakpoints as the present case (Giardino et al., 1994). However, many of the features of the proximal duplication are the same as those described for the more distal duplications. Unlike the situation with chromosome 21 where partial duplications have yielded information about critical regions for specific phenotypic findings, such fine mapping of the phenotype of 6q duplications may not be feasible. Furthermore, the present study highlights the importance of ruling out mosaicism in a parent of such an affected child, even for an abnormality that one would have presumed, a-priori, to have arisen de-novo.

**Analysis by telomere specific FISH probes of 191 karyotypically normal patients with nonspecific dysmorphic features or developmental delay, or history of multiple miscarriages.** *S.M. Jalal<sup>1</sup>, A.R. Harwood<sup>1</sup>, M.H. Anderson<sup>1</sup>, G.S. Sekhon<sup>2</sup>, R.P. Ketterling<sup>1</sup>, V.V. Michels<sup>1</sup>.* 1) Cytogenetics Laboratory, Mayo Clinic, Rochester, MN; 2) Division of Clinical Genetics, Waisman Ctr., University of Wisconsin, Madison, WI.

Complete sets of telomere specific FISH probes except the acrocentric p-arms were used from two commercial companies. Analysis was based on 147 karyotypically normal patients with non-specific dysmorphic features and/or mental retardation (NDF/MR) and 44 patients with multiple miscarriages (MM). In addition, some of these probes were also used to define breakpoint boundaries in 17 patients with abnormal karyotypes. To maintain a high mitotic index cell suspensions were dropped in a Thermotron drying chamber. At least three metaphases were analyzed by FISH for each of the 41 telomeric sites for every specimen. Among the 147 NDF/MR patients, cryptic deletions were observed in 10 (6.8%) involving 3p, 4p, 7q, 8p, 10q, 22q and Xp. An abnormality rate of 6.8% in NDF/MR patients is similar to earlier reports. Parents were available for analysis in 4/10 abnormal cases. Of these, 2/4 (50%) had a balanced translocation in one parent. No telomere region abnormality was observed by FISH in 44 individuals with MM. However, a normal polymorphic variant involving del(2)(q37.3)(D2S2986-) was observed in 9.4% of MM cases using only Cytocell™ probe set. These same patients were normal by Vysis™ probe set strongly suggesting a cryptic polymorphism of this locus. A more rare polymorphic variant involving del(7)(p22.3)(G31341-) was observed in two cases (<1%), one of whom was phenotypically normal, using both Vysis™ and Cytocell™ probes. Use of telomere specific FISH probes is effective in determination of cryptic anomalies of the telomere regions, especially for patients with NDF/MR. Individual telomere specific probes are also useful in defining breakpoint boundaries near the telomeric regions.

**Identification of two copies of large inv dup(15) chromosomes in an infant.** *B. Huang*<sup>1</sup>, *J. Bartley*<sup>2</sup>. 1) Genzyme Genetics, Orange, CA; 2) Loma Linda University, Loma Linda, CA.

Marker chromosomes originated from chromosome 15, often referred to as inv dup(15), is the most common marker chromosome found in humans. The large marker 15 that contains the Prader Willi(PWS)/Angelman syndrome(AS) chromosome region is usually associated with an abnormal phenotype of moderate to severe mental retardation, seizures, poor motor coordination, behavioral problems, and mild dysmorphic features. We report here an infant boy with two copies of the large inv dup(15).

A 10-day-old infant was found to have infantile spasm, hypotonia, lethargy and marked developmental delay. He had no distinctive dysmorphic features at time of examination. Lymphocyte chromosome analysis revealed a 48,XY,+marx2 karyotype. Fluorescence in situ hybridization with probes rRNA, D15Z, D15S11,GABRB3 showed hybridization of each probe to two locations on both marker chromosomes, demonstrating that both markers are inv dup(15) that contain PWS/AS chromosome region. Therefore, this patient has 6 copies of the PWS/AS region. To our knowledge, this is the first case reported in liveborn with two copies of the large inv dup(15). The phenotype in this patient does not appear to be significantly more severe than patients with one copy of the large inv dup(15). However, some features may not have manifested in this age and follow-up evaluation is necessary.

**Paracentric inversion of chromosome 2 [inv(2)(q21q35)] in a family with Cutis Laxa.** *M.A. Micale*<sup>1,2</sup>, *T.W. Kurczynski*<sup>1,2</sup>, *W. Curtin*<sup>3</sup>, *L. Myers*<sup>3</sup>, *M. Marcotte*<sup>3</sup>. 1) Dept Pathology; 2) Dept Pediatrics; 3) Dept Obstetrics/Gynecology, Medical Col Ohio, Toledo, OH.

Cutis laxa (CL), a connective tissue disorder involving elastin depletion or fragmentation, is characterized by an inappropriate and symmetrical looseness of skin which presents as inelastic redundant folds often involving the face, eyelids, ears, earlobes, dorsum of the hands, chest, and abdomen. The disorder may also present systemic abnormalities of the lungs, vasculature, and urinary tract, as well as prenatal and postnatal growth deficiency. CL has been shown to segregate as an autosomal dominant, autosomal recessive, or X-linked disorder. Mutations in the *ELN* gene (dominant form) at 7q11 which codes for elastin and the *ATP7A* gene (X-linked form) at Xq12-q13 have been identified. We report a multigenerational family ascertained through an amniotic fluid chromosome study performed because of an elevated MSAFP (3.4 MOM). This study revealed a paracentric inversion involving the long arm of chromosome 2 [inv(2)(q21q35)], which was subsequently found to be paternally inherited. The family history was remarkable for short stature, premature aging, and a "skin disorder" present in multiple family members. The father of the baby was diagnosed upon genetic evaluation as having CL, and the pedigree revealed that three of his four siblings (two sisters and one brother) as well as his father had short stature and a similar skin disorder. Chromosome studies performed on the unaffected six-year-old daughter revealed a 46,XX normal female karyotype while an affected sister demonstrated the inv(2q). The pregnancy was followed by serial ultrasound examinations that demonstrated generalized subcutaneous edema, an enlarged fetal abdominal circumference, and a shortened femur length. At birth, short lower extremities and loose skin at the neck, shoulders, and upper extremities were noted. The baby has had recurrent pulmonary problems requiring several admissions. Given the concordance of the abnormal phenotype and the inv(2q) in this family, it is reasonable to speculate that a gene responsible for the phenotype lies at one of the breakpoints identified in the inversion (2q21 or 2q35).

**Alternate clinical phenotypes involving deletions of HPEIII and the cri-du-chat critical region subsequent to adjacent-1 segregation of a t(5;7)(p13.3;q32.3)pat.** S.K. Manhas<sup>1,2</sup>, A. Dawson<sup>1</sup>, D. Riordan<sup>1</sup>, D. Tucker<sup>1</sup>, S. Bal<sup>1</sup>, A. Prasad<sup>2</sup>, S. Marles<sup>1,2</sup>, S.M. Nikkel<sup>1,2</sup>, C. Prasad<sup>1,2</sup>. 1) Department of Biochemistry and Medical Genetics; 2) Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada.

We present a male Cree infant born at 36 weeks gestational age with dysmorphic features to non-consanguineous parents following a normal pregnancy and normal maternal serum triple screen. Growth parameters were: birth weight 25%ile, length <3%ile, and head circumference 50%ile. Multiple anomalies noted in the infant included: hypertelorism, preauricular skin tags, tag on right cheek, short palpebral fissures, small upturned nose, Pierre-Robin Sequence, broad forehead, single palmer creases, clenched hands, short sternum, 11 ribs, cryptorchidism, small penis, right ureteral hydronephrosis, generalized hypotonia and an unusual cry. ECHO demonstrated a large ASD with poor left ventricular function. Atrial flutter with 2:1 block was present. Cranial imaging revealed agenesis of the corpus callosum and cerebellar hypoplasia. The father, with a different unrelated partner, has had three fetuses with holoprosencephaly and multiple congenital anomalies. Chromosomal analysis of this newborn revealed an unbalanced male karyotype with a derivative chromosome 5 resulting from a balanced paternal translocation. The child's karyotype was interpreted as: 46,XY,der(5)t(5;7)(p13.3;q32.3)pat. The net genetic imbalance resulting from the der(5) is monosomy and trisomy for the distal portions of chromosomes 5p and 7q respectively. The monosomy of chromosome 5p13.3@pter necessarily includes deletion of the critical region of the cri-du-chat syndrome (mapped to 5p15.2). It is likely that the first three pregnancies resulted in fetuses with holoprosencephaly subsequent to inheritance of the der(7). The net genetic imbalance resulting from the der(7) is monosomy and trisomy of chromosomes 7q and 5p respectively. Monosomy for 7q32.3@qter includes deletion of the HPEIII gene for holoprosencephaly mapped to 7q36. The clinical features of the offspring are discussed in relation to their chromosomal deletions and duplications.



**Segregation of inherited Robertsonian translocations as assessed through prenatal diagnoses.** *S. Langlois<sup>1</sup>, R.D. Wilson<sup>1,2</sup>, H. Bruyere<sup>3</sup>*. 1) Dept Medical Genetics; 2) Dept. of Obstetrics and Gynecology; 3) Dept. of Pathology, University of British Columbia, Vancouver, Canada.

**INTRODUCTION:** Robertsonian (Rob) translocations are the most common structural rearrangements seen in humans. The normal progeny of carriers of Rob translocations can either have a normal karyotype or be carriers of the Rob translocation seen in the parent. A recent publication by Pardo-Manuel de Villena et al. concluded that the inheritance of ROB chromosomes in the balanced progeny of heterozygous carriers does not conform to Mendelian ratios. Their study was based on a review of previously reported segregation data from published families and one published prenatal diagnosis study. To determine if the findings could be due to reporting bias, we analyzed the segregation of ROB translocations in our prenatal diagnosis population. Patients included in the study were all patients seen in the Medical Genetics Department for prenatal diagnosis because one of the parent was a known carrier of a non-homologous Rob translocation. Cases of Rob translocations identified at amniocentesis done for advanced maternal age or other indications were excluded from the study. **RESULTS:** A total of 31 couples were seen for prenatal diagnosis in 53 pregnancies which resulted in a normal/balanced progeny. In 37 cases, the mother was the carrier of the Rob translocation and in 16 cases the father was the carrier. 33 cases were 13;14 translocations and 10 cases were 14;21. There were two cases of 13;22, 14;15, 15;21 and 15;22. There was 1 case of 14;21 and 1 of 14;22. The segregation ratio was analyzed according to the sex of the carrier parent. Offspring of female carriers were found to inherit the balanced Rob translocation in 59% of cases whereas offspring of male carriers inherited the translocation in 50% of cases. **CONCLUSION:** Our results support the findings of Pardo-Manuel de Villena et al. and indicate that in female transmission the segregation of the Rob translocation does not conform to Mendelian ratios and is in favour of the transmission of the Rob translocation to the offspring. This results in an increased proportion of at risk individuals amongst the progeny of female carriers.

**Developmental Genome Anatomy Project: Breakpoint localization updates.** *A.W. Higgins<sup>1,6</sup>, E. Lemyre<sup>4,7</sup>, G.A.P. Bruns<sup>3,6</sup>, C. Farra<sup>1,6</sup>, J.F. Gusella<sup>2,6</sup>, B.R. Korf<sup>1,2,6</sup>, S.R. Herrick<sup>1</sup>, A.H. Ligon<sup>1,6</sup>, J. Lewis<sup>1</sup>, R.L. Maas<sup>1,6</sup>, M.E. MacDonald<sup>2,6</sup>, A.M. Michelson<sup>1,5,6</sup>, B.J. Quade<sup>1,6</sup>, C. Rooryck<sup>4,7</sup>, F. Tihy<sup>4,7</sup>, C.C. Morton<sup>1,6</sup>.* 1) Brigham & Women's Hospital, Boston, MA; 2) Massachusetts General Hospital, Boston, MA; 3) Children's Hospital, Boston, MA; 4) Hôpital Ste. Justine, Montreal, QUE; 5) Howard Hughes Medical Institute; 6) Harvard Medical School, Boston, MA; 7) University of Montreal, Montreal, QUE.

The goal of the Developmental Genome Anatomy Project (DGAP) is to identify and study genes critical in human development by analyzing samples from patients with balanced chromosomal rearrangements and at least one congenital anomaly. We hypothesize that in a substantial proportion of these patients a developmentally important gene will be disrupted or dysregulated by the breakpoint and be etiologic in the abnormal phenotype. DGAP involves three steps: FISH mapping of the breakpoints using BACs at ~1 Mb intervals; sequence analysis and candidate gene identification in the region of the breakpoints; and functional analysis of candidate genes in model organisms (mouse and *Drosophila*). We have established a growing network of collaborators among clinical cytogeneticists, clinical geneticists and genetic counselors around the U.S. and abroad and now have over 90 cases in our database from patients with a variety of developmental defects and apparently balanced rearrangements. According to a prioritization scheme, we have FISH-mapped 10 breakpoints at the highest resolution possible using a genomic BAC map with BACs FISH mapped at ~1 Mb intervals. Two of these 10 breakpoints have been localized to single BACs, RP11-781L3 and CTD-3193O13, and narrowed to ~40 kb and ~20 kb intervals within these clones, respectively. These cases are now undergoing detailed molecular analyses. Three additional breakpoints have been mapped between 2 genomic BAC clones within intervals of ~90 kb, ~100 kb, and ~200kb. These FISH data along with clinical and cytogenetic data for each case can be found at the DGAP website (<http://dgap.harvard.edu>). As DGAP expands and progresses, we believe this will be a powerful resource for scientists in the genetics and developmental biology communities.

**Prenatal diagnosis of a de novo marker chromosome using FISH and CGH.** A. Zaslav<sup>1</sup>, B. Levy<sup>2</sup>, J. Jacob<sup>1</sup>, D. Blumenthal<sup>3</sup>, E. Valdarrama<sup>3</sup>, J.E. Fox<sup>3</sup>. 1) Department of Pathology, Long Island Jewish Medical Center, The Long Island Campus of the Albert Einstein College of Medicine, New Hyde Park, NY; 2) Departments of Human Genetics & Pediatrics, Mount Sinai School of Medicine, New York, NY; 3) Department of Pediatrics, Schneider Childrens Hospital, Long Island Jewish Medical Center, The Long Island Campus of the Albert Einstein College of Medicine, New Hyde Park, NY.

Prenatal cases with de novo marker chromosomes are problematic because of the sparsity of information regarding the phenotypic consequences to the fetus. We report a prenatal case of mosaicism for a marker chromosome that was characterized using FISH and Comparative Genomic Hybridization (CGH). Amniocentesis was performed on a 43 year old, white female for AMA. The karyotype was 47,XY,+mar[13]/46,XY[21]. Chromosome analysis of 100 cells from each of the parents revealed normal chromosomes. A high-resolution ultrasound at 16.6 weeks revealed that the fetus had clenched fists. The AneuVysion TM Probe set (Vysis, Inc, Downers Grove, IL) encompassing the centromeric regions of chromosomes 13, 18, 21, X, and Y was used. The marker chromosome demonstrated a positive signal for chromosome 18. CGH was also performed to more accurately characterize the size and breakpoints of the marker chromosome. CGH demonstrated a gain of the pericentric region of chromosome 18 from q10 to q12. Thus, the fetus was partially trisomic for chromosome 18 and the revised karyotype was 47,XY,+mar(18)[13]/46,XY[21].rev ish enh(18)(q10q12). Based on these findings the patient terminated the pregnancy. Autopsy performed on fragmented placenta and fetal parts revealed only a high arched palate. Follow-up cytogenetic studies on fetal tissue confirmed the above findings. This report illustrates the importance of using molecular cytogenetic techniques to determine the origin and also the size and breakpoints of de novo prenatally diagnosed marker chromosomes. This information, together with the clinical data, is important for genetic counseling issues. In addition, it provides insight into phenotype/genotype correlations.

**Phenotypic and cytogenetic analysis of a liveborn infant with prenatally detected trisomy 9p and partial trisomy 8p resulting from 3:1 malsegregation of a maternal reciprocal translocation.** *R. Smith<sup>1</sup>, H.E. Wyandt<sup>2</sup>, J.M.*

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Tertiary or double trisomy, is a rare occurrence, produced by 3:1 malsegregation of a reciprocal translocation. We present an infant with trisomy 9p and partial trisomy 8p as a result of such an event. Our patient was born to a 37 y.o. G4P0 mother with a history of 3 previous losses. Maternal karyotyping revealed a 46,XX,t(8;9)(p22;q12) balanced translocation. Prenatal ultrasound revealed a VSD and a small aortic root, a unilateral CPC, and a slightly small cerebellum. Amnio confirmed a 47,XX,+der(9),t(8;9)(p22;q12) unbalanced karyotype.

The infant was born AGA at 35 4/7 wks due to PROM. Features noted on exam included: broad and prominent nasal bridge, short distinct philtrum, prominent upper lip with downturned corners of the mouth, overfolded helices with prominent antihelix, redundant nuchal skin, close set nipples, and digital anomalies consisting of nail hypoplasia with distal thumb hypoplasia and 5th finger clinodactyly. An echocardiogram revealed complex congenital heart disease consisting of pre-ductal coarctation of the aorta, two VSDs, small secundum ASD, and dysplastic aortic and pulmonic valves. Head MRI showed mild prominence of the temporal horn of the left ventricle and a slightly small cerebellar vermis. The infant also had 3rd degree heart block. Support was electively withdrawn at 1 week of life.

Our patient's features appear to primarily resemble those of trisomy 9p, although congenital heart defects can also be seen in partial trisomy 8p. Tertiary trisomies, typically of maternal origin, most frequently occur with translocations involving acrocentrics or chromosome 9, where the partial trisomies of the chromosome segment(s) are small enough to be viable. Our case supports this finding and illustrates the need to consider the phenotypic consequences of all possible segregation products when counseling couples where the mother carries a reciprocal translocation involving chromosome 9.

**Deletion Mapping in a Normal Father and a Normal Child Reveals Suspected Chromosome Instability at**

**21q11.2.** *H. Tinkel<sup>1</sup>, S. Finkernagel<sup>1</sup>, F. Desposito<sup>2</sup>, C. Pittore<sup>3</sup>, K. Reynolds<sup>1</sup>, L. Sciorra<sup>1</sup>.* 1) Department of Obstetrics and Gynecology, RWJUH, UMDNJ, New Brunswick, NJ; 2) Department of Pediatrics, New Jersey Medical School, UMDNJ, Newark NJ; 3) Division of Genetics, Mercer Medical Center, Trenton, NJ.

We present a child with speech delay and an otherwise normal phenotype with a deletion of chromosome 21q11.2 to 21q21. The child's father, who has a normal phenotype, was found to have a smaller deletion on 21q11.2 in 50% of his cells with the remaining cells normal.

The patient, a two year and eight month old girl, is the second child of healthy, unrelated parents. Testing has shown mostly age-appropriate milestones. However, she has delayed speech and began to speak beginning at two years of age. The patient is thought to have apraxia with a discrepancy between receptive language, which is judged to be four months above her chronological age, and expressive language. Physical examination is normal revealing no dysmorphism.

Metaphase spreads were analyzed via FISH with YAC DNA inserts spanning chromosome 21q11.1 to 21q21. The YACs in order from proximal to distal are 831b6, 52h5, 849b10, 949b9, 881d2, 746b10, 937e2, 940e2, 916h7, 925h10, and 799g3. The subjects were also analyzed with marker D21S13 located approximately on YAC 881D2.

The child was shown to be deleted in all cells for YACs 881d2, 746b10, 937e12, 940e2, 916h7 and 925h10. The father was found to be deleted in 50% of his cells only for marker D21S13.

These findings support previous work showing that proximal chromosome 21q does not include genes that are responsible for severe clinical defects. This pattern of inheritance also suggests a region of instability in chromosome 21q. We postulate that the region of deleted material in the father's chromosome created structural difficulties in the alignment of the chromosome 21s during meiosis resulting in the further loss of genetic material seen in the daughter. To our knowledge, this is the only report of such a pattern.

**Cytogenetics studies including FISH for 15q11.2/17p12 duplications and subtelomeric rearrangements in children with autistic spectrum disorder.** *R.T. Zori<sup>1</sup>, B. Gray<sup>1</sup>, K. Keller<sup>1</sup>, P. Wharton<sup>2</sup>, A. Bent-Williams<sup>1</sup>, A. Ward<sup>1</sup>, H. Stalker<sup>1</sup>, M. Paulk<sup>2</sup>, C.A. Williams<sup>1</sup>. 1) R.C. Philips Unit, Division of Genetics, Department of Pediatrics, University of Florida, Gainesville, FL; 2) Jacksonville Center for Autism and Related Disabilities, Department of Pediatrics, University of Florida, Gainesville, FL.*

To assess the frequency of chromosome aberrations 50 children with an autistic spectrum disorder were identified. Age range of the children was 2.6 to 20.0 years (av=8.3). M:F ratio was 2.6:1. Diagnoses included classic infantile autism (39%), pervasive developmental disorder, not otherwise specified (48%) and Asperger syndrome (14%). All 50 children were studied for chromosome abnormalities by routine G-banded blood chromosome analyses and by FISH analyses for 15q11.2 duplications, 17p11.2 duplications, and subtelomeric rearrangements. A maternally inherited chromosome 5 short arm duplication was detected in one child by routine chromosome analysis and an interstitial duplication of the Prader-Willi and Angelman syndrome critical region of chromosome 15 was detected by FISH analysis in another child. No instances of 17p11.2 duplication or of any chromosomal subtelomeric rearrangements were observed. Detection of these two cases underscores the importance of obtaining routine chromosome analyses and 15q11.2 FISH analyses in children with autistic spectrum disorder. Although subtelomeric FISH studies may be useful in the diagnostic assessment of certain subgroups of children with mental retardation syndrome, our results do not support their routine use in the diagnostic assessment of children with autism.

**Interphase FISH defines characteristic chromosomal abnormalities in diffuse large B-cell lymphomas. D.**

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Complex karyotypic alterations are characteristic of Diffuse Large B-Cell Lymphomas (DLBCL), the most common subtype of non-Hodgkin's Lymphomas (NHL). Using Multicolor-Fluorescence in situ Hybridization (M-FISH) we resolved complex chromosomal changes and delineated specific regions that were recurrently involved in rearrangements. These included 1p36, 3p21, 3p24, 3q27, 6q21, 8q24, 14q32, 17p13, and 18q21. To determine possible alterations in these regions specifically among cases without cytogenetic studies on their diagnostic specimen, we examined whole nuclei extracted from paraffin embedded tissue cores using interphase FISH studies with cosmid, YAC, and/or BAC probes. Twenty-nine (88%) of the 33 DLBCL cases analyzed were abnormal as determined by interphase FISH and 13 (40%) of these exhibited disruptions of IgH. Rearrangements of bcl2, including fusion with IgH and amplification were observed in 8 (24%) cases; the bcl6 alterations and c-myc amplification were found in 3 cases each (9%). Loss of heterozygosity of p53 (17p13) and p58 (1p36) were observed in 9 (27%) and 6 (18%) cases, respectively; while loss of 3p24 and 3p21 were observed in 8 (24%) and 5 (15%), respectively. Nine (27%) cases exhibited complex abnormalities involving 2 or more abnormal clones frequently with increasing ploidy. Seventeen (51%) cases contained the rearrangements of bcl2, bcl6, c-myc and/or IgH. Ten (30%) of these showed multiple copies of fusion signals or amplification of bcl2, bcl6 or c-myc. Fourteen (42%) cases revealed the loss of one or more regions investigated, with 7 of them showing loss of multiple regions. Nine of these 14 cases showed no rearrangement of the bcl2, bcl6, c-myc or IgH, indicating the possible presence of a primary abnormality other than that investigated. Interphase FISH using a panel of probes has been a valuable tool to assess chromosomal abnormalities of interest and has demonstrated that various combinations of the primary and secondary abnormalities may be present in DLBCL, each resulting in a distinct subset of this histological subtype of lymphoma.

**The Common and Often Cryptic 1p36 Deletion.** *K.K. Phillips, J.H. Tepperberg, P. Stone, D.J. Holliday, I.K. Gadi, P.N. Mowrey, P.R. Papenhausen.* Dept Cytogenetics, Lab Corp of America, Res Triangle Pk, NC.

Monosomy 1p36 is reported to have an incidence of ~1 in 10,000. The breakpoint within the p36 band (i.e., the size of the deletion) varies, and this generally correlates with phenotypic variability, such that patients with larger deletions are more severely affected. Severe phenotypic features include failure to thrive, seizures, heart malformations, dysmorphic facies, and development of mild to severe mental retardation. Milder features can be limited to generalized muscular hypotonia. In our laboratory, we routinely follow up apparent de novo 1p36 deletion cases with FISH subtelomere probe analysis (Vysis, Inc.), both to verify deletion status and to allow resolution of possible cryptic reciprocal translocations. Until it became commercially unavailable, we also used the more proximal p58 FISH probe (Oncor, Inc.) to permit estimation of the size of the deletion. We have identified 8 patients (3 prenatal and 5 postnatal) in the last ten months with isolated deletion of 1p36. An additional prenatal case, referred for maternal serum DS risk without ultrasound abnormalities, was initially reported as deletion 1p36 but was later found to be a cryptic balanced translocation. Follow up FISH analysis on this prenatal case, using 1p subtelomere probe, showed signal on distal chromosome 19p. Parental G-band analysis revealed that the translocation was maternal in origin, and therefore, of negligible risk to the fetus. Without the FISH data and parental analysis follow up, the case would have been initially reported incorrectly. These data indicate that 1) the incidence of monosomy 1p36 may be higher than previously reported, and 2) parental karyotype analysis and utilization of FISH should be routinely employed as precautionary follow up procedures in cases with apparent de novo 1p deletion.



**Cell lineage confined mosaicism in specimens of spontaneous abortion.** *D.K. Kalousek<sup>1,2</sup>, T.A. Loukianova<sup>1</sup>, S.S. Tang<sup>1</sup>, T. Philipp<sup>3</sup>.* 1) Dept Path/Lab Med, Univ of BC, Vancouver, BC, Canada; 2) Dept Path, Women's and Children's Health Center of BC, Vancouver, BC, Canada; 3) Clinical Obstetrics and Gynecology Donauespital im SMZ-Ost, Vienna, Austria.

High incidence of chromosomal mosaicism is described in preimplantation embryos, while the frequency of fetal mosaicism detected by amniotic fluid culture is less than 0.5%. Confined placental mosaicism (CPM) is recorded in 1-2 % of pregnancies examined by CVS at 10-12 weeks of gestation. The mechanism by which the high levels of preimplantation mosaicism are reduced during the first trimester are not understood and the role of mosaicism in early pregnancy loss has been questioned. Previous studies of spontaneous abortions (SAs) using tissue culture and traditional cytogenetic techniques describe the frequency of mosaicism at around 10 % (Warburton et al, 1978). We have used comparative genomic hybridization (CGH) analyses for detection of cell lineage confined mosaicism in 78 specimens from first and second trimester SAs. CGH analysis of trophoblast, chorionic stroma and amnion revealed 11 specimens with cell lineage confined mosaicism from the first trimester (11 out of 43) and one from the second trimester (1 out of 25). The most common mosaicism (6 cases) involved the detection of aneuploidy (trisomy 3, trisomy 15, trisomy 16, monosomy X x3) in trophoblast and chorionic stroma but a normal diploid karyotype in amnion. In two cases, triploidy was found in all cell lineages, but in amnion the additional sex chromosome duplication was presented (70,XXXYY). Three sex chromosome aneuploidies confined to trophoblast (monosomy X x2; 47,XXY) in otherwise diploid conception were diagnosed. The CGH analysis of all cases was complemented with either flow cytometry, traditional cytogenetic analysis or fluorescence in situ analysis. This study of 43 first trimester SA specimens showed a 25% frequency of mosaicism confined to a specific cell lineage. This result suggests a significant role for chromosomal mosaicism in early pregnancy loss.

**Paternal UPD14 is responsible for a distinctive malformation complex.** *K. Kurosawa<sup>1</sup>, H. Sasaki<sup>2</sup>, Y. Sato<sup>1</sup>, M. Yamanaka<sup>1</sup>, M. Shimizu<sup>3</sup>, Y. Ito<sup>4</sup>, T. Okuyama<sup>4</sup>, M. Matsuo<sup>1</sup>, K. Imaizumi<sup>1</sup>, Y. Kuroki<sup>1</sup>, G. Nishimura<sup>5</sup>.* 1) Kanagawa Children's Med Ctr, Yokohama, Japan; 2) Department of Human Genetics, National Institute of Genetics, Japan; 3) Tokyo Metropolitan Bokuto Hospital, Japan; 4) National Childrens Hospital, Japan; 5) Division of Radiology, Nasu-chuo Hospital, Japan.

In humans, uniparental disomy (UPD) is known as the cause of several malformation syndromes. To date, 23 cases with maternal UPD14 (matUPD14) and 4 cases with paternal UPD14 (patUPD14) have been reported. Most cases with matUPD14 and all cases with patUPD14 had translocated ch14. MatUPD14 cases did not compile a distinctive pattern of malformations, but patUPD14 cases may have constituted a distinctive malformation syndrome. We report three additional patUPD14 cases, all of which shared common manifestations with those of previously reported cases, which unequivocally established that patUPD14 gives rise to a distinctive malformation syndrome. Moreover, two of 3 cases, unlike previously reported cases, had a normal karyotype, which may suggest that patUPD14 is more common than previously thought and the majority of cases have gone unrecognized. The constellation of thoracic hypoplasia with short, wavy ribs, diastasis recti, and minor facial dysmorphism. A total of 15 highly polymorphic microsatellite markers spanning the entire long arm revealed patUPD14 for all patients. Human ch14 has a large region homologous to mouse ch12. Mouse embryos with patUPD12 display skeletal defects, all of which are consistent with the phenotype of human patUPD14. It is therefore likely that the malformations common to human patUPD14 and mouse patUPD12 result from an evolutionarily conserved imprinted gene(s). Recently, two imprinted genes, *Gtl2* and *Dlk1*, have been identified in the distal regions of mouse ch12 and their orthologues on human ch14. These genes may be candidates for the patUPD14 phenotype. In addition, the sheep callipyge (CLPG) phenotype shows hereditary muscular hypertrophy with an unusual parent-of-origin effect, and the gene is mapped to the *Gtl2/Dlk1* locus. The complex roles of the CLPG mutation raises the assumption of the important role of *Gtl2/Dlk1* locus in the development of the patUPD14 and matUPD14 phenotypes.

**Chromosome 22q11.2 Deletion Syndrome: FISH Study of 71 Cases.** *S.J. Abulhasan, S.A. Al-Awadi.* Kuwait Medical Genetics Centre, Maternity Hospital, P.O. Box 31121, Sulaibikhat 80901, Kuwait.

Chromosome 22q11.2 deletion syndrome, which includes DiGeorge (DGS), velocardiofacial (VCFS), conotruncal anomaly face (CTF), and recently Kenny Caffey (KCS) syndrome is the most common microdeletion syndrome. It has been estimated that deletion at 22q11.2 occur in 1:4000 newborns and in up to 90% of patients with DGS and VCFS. The majority of deleted patients share a common 3 Mb hemizygous deletion of 22q11.2. This study reports FISH results of 71 cases presented clinically with a spectrum covering the above mentioned syndromes. FISH technique applied to blood lymphocyte metaphase spreads and interphase nuclei using dual color DNA probe specific for DiGeorge critical region (DGCR) on chromosome 22q11.2 and to the band 22q13 as a control region (Vysis) and probe D22S75 (Oncor), revealed 22 q11.2 haploinsufficiency in 19 cases (27%). Seven cases (37 %) inherited the deletion from either parent (maternal in 5 and paternal in 2). Five parents had translocation involving chromosome 22q and other non-homologous chromosome. Two maternal deletions were in the mosaic form so pointing out to the possibility of gonadal mosaicism as a cause of 22q11.2 deletion in the affected offspring. The 7 parents with the deletion were phenotypically normal except one who had congenital heart disease and 22q11.2 deletion. Parents with 22q11.2 deletion who have a 50% risk of transmitting the deletion to their offspring have to perform amniocentesis for prenatal diagnosis and preimplantation genetic diagnosis using FISH technique whenever possible.

**Rapid sequence-based definition of chromosomal abnormalities.** *P.M. Cazcarro, P.K. Rogan, J.H.M. Knoll.* Medical Genetics & Mol Medicine, Children's Mercy Hospital, Kansas City, MO.

Chromosomal rearrangements are generally delineated by in situ hybridization using large recombinant DNA probes. The sequences of these probes are often not precisely determined, and their size precludes detection of small deletions, duplications or cryptic abnormalities within corresponding or closely related genomic sequences. Nevertheless, they have been useful in demonstrating heterogeneous breakpoints and shortest regions of overlap in disease states, which has led to assignment of chromosomal intervals to specific phenotypes in many disorders. We developed custom single copy (sc) probes from the draft genome sequence for fluorescence in situ hybridization (scFISH; *Genome Res.* 11:1086, 2001), and now use this approach to precisely delineate chromosome abnormalities at a resolution equivalent to genomic Southern analysis. Probes, 1.5 - 10 kb in length, have been designed, produced by long PCR, visualized and validated rapidly as this approach does not require cloning. We have validated 75 probes from 23 chromosomal disease intervals. We demonstrate the versatility and sensitivity of this approach by presenting examples of (A) deletion breakpoint definition in Smith-Magenis syndrome on chromosome 17p11.2 (eg. for the *FLII* gene: probes from IVS9-IVS12, IVS12-IVS14, and IVS15-Ex21; for *MFAP4*: IVS2-3UTR; for *SHMT1*: IVS1; for *LLGL*: promoter-exon 2, and promoter-IVS1; and for *ADORA2B*: Promoter-IVS1 and IVS1), (B) microdeletion detection at or near the chromosome 15q11.2 imprinting centers in Prader-Willi and Angelman syndromes (eg. for *IC/SNRPN*: IVS3'-exon u1B\*, and exon u5-IVS3'; for *SNRPN*, promoter-IVS1; and for *UBE3A*: IVS8-IVS9), and (C) translocation 9;22 breakpoint delineation in chronic myelogenous leukemia (eg. for *ABL1*: exon 1B-IVS1B, IVS3[3], IVS4-IVS6[2], exon 11-IVS11; for *BCR*: proximal of M-BCR[2] and IVS8). By combining sequence-based probe design with FISH, nearly any euchromatic abnormality can be readily characterized. scFISH will facilitate and refine cytogenetic studies, such as phenotype-genotype correlation, mechanisms of formation, and analyses of chromatin structure.

**Application of the complete human multicolor banding (MCB) set in cancer and clinical cytogenetics.** *T. Liehr<sup>1</sup>, A. Heller<sup>1</sup>, H. Starke<sup>1</sup>, A. Weise<sup>1</sup>, K. Mrasek<sup>1</sup>, V. Trifonov<sup>2</sup>, N. Rubtsov<sup>2</sup>, U. Claussen<sup>1</sup>.* 1) Inst. of Human Genetics and Anthropology, Jena, Germany; 2) Inst. of Cytology and Genetics, Novosibirsk, Russia.

The multicolor-banding (MCB) technique allows the differentiation of chromosome (chr) region specific areas at the band and sub-band level and is based on regionspecific microdissection libraries producing changing fluorescence intensity ratios along the chrs. The latter are used to assign different pseudocolors to specific chromosomal regions. We present for the first time the complete set of 138 regionspecific microdissection libraries for the entire human genome and the resulting MCB patterns for all human chrs at the 400 band level. More than 120 cases with congenital or acquired complex chromosomal rearrangements involving different chrs have been analyzed up to now using this DNA-based high resolution banding technique. In most of the studied cases neither GTG-banding nor 24-color-FISH could resolve the complex changes, e.g. interstitial deletions, insertions or inverted insertion. Additionally, Zoo-FISH experiments on Gorilla gorilla (GGO) and Hylobates lar (HLA) have been done using the complete human MCB probe set. In GGO no paracentric inversion was found on GGO 14, and no pericentric inversions could be demonstrated on GGO 16 or 17. In HLA the position of centromeres, the breakpoints and the up to now unknown orientation of small "rearranged" chromosomal regions could be determined. Moreover, interphase analysis using the MCB-probe sets for chr 2 and 5 showed, that interphase chrs present with a banding pattern similar to that of metaphase chrs at any stage of the cell cycle. In summary, the MCB-technique is a high resolution alternative to other M-FISH based banding approaches and suited to clarify within one single FISH experiment, which changes appeared in complex rearrangements, to study in detail the architecture of the interphase nucleus and to do high-resolution comparative ZOO-FISH studies. Acknowledgments: Supported in parts by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the DAAD, the DFG, the EU and the Wilhelm Sander-Stiftung.

**Human multicolor banding (MCB) probes applied for ZOO-FISH in Gorilla gorilla and Hylobates lar.** A. Heller<sup>1</sup>, K. Mrasek<sup>1</sup>, M. Rocchi<sup>2</sup>, V. Trifonov<sup>3</sup>, N. Rubtsov<sup>3</sup>, H. Starke<sup>1</sup>, U. Claussen<sup>1</sup>, T. Liehr<sup>1</sup>. 1) Inst. of Human Genetics and Anthropology, Jena, Germany; 2) Institute of Genetics, Bari, Italy; 3) Inst. of Cytology and Genetics, Novosibirsk, Russia.

The origin of human and ape chromosomes has been studied by comparative chromosome-banding analysis and more recently by fluorescence in situ hybridization (FISH) studies using human whole chromosome painting (wcp) probes. Using these techniques, however, it is not always possible to determine the exact breakpoints or orientation of specific DNA-regions which have been distributed on different chromosomes in apes compared to human. To overcome that problem, in the present study the recently developed multicolor banding (MCB) probe set for all human (HSA) chromosomes has been applied to reanalyze the chromosomes of Gorilla gorilla (GGO) and Hylobates lar (HLA). These two species have been chosen, as karyotype of the first one is very similar to the human and the second one is known to be highly rearranged with respect to the human one. The MCB results for GGO agree with those of most previous banding and FISH studies, however, the breakpoints for the pericentric inversion on GGO 3 were defined more precisely, no paracentric inversion was found on GGO 14, and no pericentric inversions could be demonstrated on GGO 16 or 17. Former FISH studies using human wcp probes clarified, which HLA chromosomes are homologue to which human ones. Nevertheless, using MCB it could e.g. be shown that material of HSA 8 is present not only on HLA 7 and 9 but also on 5 and 12. The orientation of chromosomal subregions in HLA have been unclear up to present. Using MCB it could e.g. be shown that HLA 19 is homologous to 1q44-1q31::1p31.1-1p34.2::1q32-1q44. We could demonstrate, that MCB leads to complete information about all kinds of rearrangements which appear during primate chromosomal evolution. Acknowledgments: Supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the EU and the Wilhelm Sander-Stiftung.

**Singlet/doublet FISH patterns point out allele-specific differences, but not necessarily asynchronous replication.**

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Asynchronous replication is considered a distinct feature of particular genes and is commonly associated with allele-specific methylation and monoallelic expression. In imprinted genes, this difference is fixed in a parent of origin specific fashion, whereas in X-linked and certain immune system-associated, growth factor, and receptor genes, doublets and singlets are originally randomly distributed. Allelic exclusion and clonal expansion may eventually lead to a seemingly nonrandom distribution. The asynchronous replication behavior of such genes is usually analyzed with FISH by determining the singlet/doublet ratio of the two alleles. Such studies are based on the assumption that unreplicated loci generate single hybridization signals, whereas replicated ones produce doublets. Of interest, however, the absolute numbers of cell with asynchronous FISH patterns do not differ significantly in S- and non-S phase cell fractions. To investigate this issue further, we therefore generated dual-color labeled RNA FISH probes from the imprinted Prader-Willi region that bind to nonoverlapping solitary DNA strands. By that, we clearly distinguish between doublets in replicated (two chromatids with two dual-colored signals) and in nonreplicated regions (one chromatid with two mono-colored signals). The results of our experiments prove that the vast majority of doublets actually do not represent replicated DNA. Differences in the compaction and utilization of the two nonreplicated alleles may lead to a better separation of the DNA strands of the allele with two mono-colored signals. In case this notion is true, this FISH pattern should be strongly influenced by the respective preparation and denaturation methods. Indeed, we found that the number of asynchronous pattern significantly increases in halo preparations of unstimulated G0 mononuclear cells, whereas this is not the case in those of PHA-stimulated cells.

**A case with persistent low mosaicism of trisomy 18 detected by interphase FISH: an artifact or true mosaicism?**

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Prenatal diagnosis using interphase fluorescence in situ hybridization (FISH) on uncultured amniotic fluid cells is generally considered accurate and reliable. However, detection of mosaic chromosome aneuploidy, in particular at a low level without confirmation by standard cytogenetics, can be problematic for result interpretation and genetic counseling. We report here prenatal detection by interphase FISH and postnatal follow up of a case with mosaic trisomy 18. Amniocentesis and interphase FISH were performed for a 34-year-old G3P1->2 woman at 22.6 weeks gestation. U/S showed 3mm unilateral choroid plexus cyst. Cytogenetic study demonstrated a normal male karyotype (46,XY) in 27 colonies. Interphase FISH (AneuVysion, Vysis, Inc.) revealed an X and a Y and three signals for chromosome 18 in 12% (12/100) of cells. Probes for chromosomes 13 and 21 showed a normal pattern. The FISH result was reported as inconclusive. The patient decided to continue the pregnancy. A foreskin biopsy sample was obtained after a normal delivery. Cytogenetic analysis showed a normal male karyotype in 20 cells examined. Interphase FISH using the same chromosome 18 probe revealed three signals in 6% (6/100) and 11% (11/100) of cells from two independent touch preps. FISH on the cultured cells showed a similar result: 5% (10/200) of cells with three signals for 18. The FISH results raise a concern for a low mosaicism of trisomy 18. Further evaluation of a buccal smear and a urine sample by interphase FISH showed 17% (17/100) of buccal cells and 12% (19/165) of urine cells with three signals for chromosome 18. Clinical evaluation at 6 weeks of age revealed normal development with no dysmorphic features and no significant congenital anomalies. The persistent presence of an abnormal signal pattern for chromosome 18 in various tissues is highly suggestive of a true mosaic trisomy 18. However, without confirmation by standard cytogenetics, a rare artifact or polymorphism cannot be totally ruled out.



**Referral trends for FISH analysis on blood samples in a regional cytogenetics laboratory over a 9 year period.** *E. Mak-Tam, A. Mar-Wah, P. Wyatt.* Dept. Genetics, North York General Hospital, North York, ON, Canada.

The Cytogenetics database was reviewed to look at the requests for blood FISH analysis over a 9 year period from 1992 to 2000. This regional laboratory accepts samples from a wide geographic region and from associate clinics. The percentage of bloods referred for FISH analysis increased from 1.5% in 1992 to an average of 10.5% in the last 5 years. Of the total bloods with FISH analysis, over 80% were for microdeletion probes. The most frequent referrals were for the William and DiGeorge/Velocardiofacial probes. Other microdeletion probes included Smith-Magenis, Prader Willi/Angelman and STS probes. The positive (detection) rate interestingly, decreased from a high of 25% to an average of 6.5%, which has been stable for the last 5 years. The initial high detection rate confirmed appropriate referrals from our own geneticists during the time when microdeletion probes were first introduced. FISH tests can now be ordered by any clinician. With increased availability of new probes, the clinician can make a diagnosis previously not possible with routine classical cytogenetics. However, added laboratory costs and patient follow up are real concerns. Monitoring detection rates and referral patterns can be important components of an effective laboratory quality assurance program.

**Visualization of multiple peptide nucleic acid (PNA) probes by combinatorial multicolor fluorescence in situ hybridization.** *K.L. Taneja<sup>1</sup>, B. Williams<sup>1</sup>, E.A. Chavez<sup>2</sup>, J. Coull<sup>1</sup>, R.H. Singer<sup>3</sup>, P.M. Lansdorp<sup>2</sup>*. 1) Boston Probes, Inc, Bedford, MA; 2) Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC, Canada; 3) Departments of Anatomy, Albert Einstein College of Medicine, Bronx, New York 10461.

Combinatorial labeling of probes (i.e., with two or more different reporters) increases the number of target sequences that can be detected simultaneously by fluorescence in situ hybridization. We have designed and developed directly labeled PNA probes to distinguish up to  $2^n - 1$  probes ( $n$  is the number of different fluorochromes) using an epifluorescence microscope equipped with a digital imaging camera and computer software for pseudocoloring and merging images. Peptide nucleic acids (PNA) are synthetic mimics of DNA in which the phosphodiester backbone has been replaced with 2-aminoethyl glycine linkages, but maintaining the four natural nucleobases. PNA probes bind to the complementary DNA sequence obeying Watson-Crick base pairing, however the neutral backbone of the PNA molecule allows for the PNA/DNA binding to occur more rapidly and more tightly than DNA/DNA binding. Chromosome specific composite PNA probe sets were generated from the human satellite sequences, in which the different fluorochromes were incorporated. The technique has been used to delineate the centromeres or satellite regions of different human chromosomes, on both 4',6-diamidino-2-phenylindole-stained metaphase spreads and interphase nuclei in a single hybridization experiment and to detect and to enumerate chromosomes. Our data suggest that multiplex fluorescence in situ hybridization (M-FISH) could have wide clinical utility, particularly in detection and enumeration of chromosomes in a given sample. Multi-parameter hybridization analysis should facilitate the study in molecular cytogenetics and probe-based diagnosis of pathogens.

**Duplicated genes and markers in 17q11.2 detected by high resolution FISH and involvement of repeated regions in NF1 microdeletion breakpoints.** *P. Riva<sup>1</sup>, C. Gervasini<sup>1</sup>, M. Venturin<sup>1</sup>, A. Bentivegna<sup>1</sup>, L. Corrado<sup>1</sup>, M. Stabile<sup>2</sup>, M. Clementi<sup>3</sup>, R. Tenconi<sup>3</sup>, L. Larizza<sup>1</sup>.* 1) Dept Biol & Genetics, Univ Milan, Milan, Italy; 2) Medical Genetic Service, Cardarelli Hospital, Naples, Italy; 3) Dept. of Pediatrics, University of Padua, Italy.

Evidence links duplicons flanking the NF1 gene in 17q11.2 to the breakpoints of NF1 microdeletion syndrome. Physical mapping and structural definition of duplicated regions is often precluded by using conventional approaches, thus the sequence ready map of 17q11.2 and numerous other pericentromeric regions is still a draft. Recently by using an alternative physical mapping approach based on high resolution FISH on stretched chromosomes and DNA fibers, we detected and mapped duplications of BLMH and GOS28 genes and SHGC30113 transcript in 17q11.2. We found further duplications involving ESTs AN2, 41099, M79255 and IB518, anchored to 41c23, not reported as duplicated in 17q11.2 physical maps. To disclose the genomic organization of duplicated loci we mapped, directly by fiber FISH, 41c23 in respect to other genomic clones establishing their reciprocal order: cen-41c23-977d19-1015j22-gap-977d19-1015j22-41c23-tel. A parallel study aimed at verifying the involvement of repeated sequences in the deletion bkp of NF1 patients is also in progress. We used cell lines from 5 NF1 deleted patients (pts) from a group of 25 pts previously characterized by conventional FISH. In all cases we identified a centromeric bkp flanking D17S1317, while the telomeric bkp flanks ACCN1 gene in 50% of pts. By using FISH with selected PACs, targeting the regions adjacent at the centromeric and telomeric bkps, overlapping signals were detected on stretched deleted chromosomes. FISH on DNA fiber from three pts allowed to observe that signals given by PACs anchored to D1S1317 and to MCP-3 in one pt, as well as D1S1317 and ACCN1 in two pts, were partially overlapping indicating the presence of common sequences located 1-3 Mb apart on the normal chromosome 17. Use of high resolution FISH is a powerful tool of mapping duplicated regions. Data disclosing 17q11.2 genome organization will allow to clarify NF1 microdeletion syndrome pathogenetic mechanism.

**mRad54 is a key protein in the maternal repair of ionizing radiation-induced sperm lesions.** *F. Marchetti<sup>1</sup>, J. Essers<sup>2</sup>, R. Kanaar<sup>2</sup>, A.J. Wyrobek<sup>1</sup>*. 1) BBRP, Lawrence Livermore National Laboratory, Livermore, CA; 2) Erasmus University, Rotterdam, The Netherlands.

The repair of sperm DNA lesions is essential for genomic integrity of the zygote and proper embryonic development and is thought to be controlled by maternal mRNAs and proteins stored in the egg before fertilization. We investigated the role of mRad54 in the repair of ionizing radiation (IR)-induced sperm DNA lesions using female mice carrying a knockout mutation of this gene. B6C3F1 males were treated with 0 or 4 Gy gamma irradiation and were mated with either B6C3F1 or mRad54 null females seven days after exposure. First-cleavage (1-C1) zygote metaphases were analyzed for the presence of chromosomal aberrations using chromosome painting. The baseline frequencies of fertilized eggs (fertilization rate), 1-C1 metaphases (zygotic development) and chromosomal aberrations were not different between B6C3F1 and mRad54 null females. Paternal exposure to IR did not reduce the fertilization rate and zygotic development among B6C3F1 females. Among mRad54 null females, the fertilization rate was also unaffected, however, there was a significant reduction in the number of 1-C1 metaphases (75% vs 55%,  $P=0.02$ ). As expected, paternal exposure to IR increased the frequencies of zygotes with chromosomal aberrations in both B6C3F1 and mRad54 null females ( $P<0.001$ ). However, in mRad54 null females there was a 24% increase in the frequencies of zygotes with chromosomal aberrations with respect to B6C3F1 females. Additionally, there was a significant 2-fold increase ( $P=0.004$ ) in the total number of chromosomal aberrations per metaphase analyzed. This was mostly due to a 7-fold increase in the frequencies of chromatid-type aberrations. These results suggest that mRad54 is a key protein in the biochemical processing and repair of IR-induced sperm DNA lesions during the first cell cycle of mouse development. Work performed under the auspices of the U.S. DOE by the University of California, Lawrence Livermore National Laboratory under contract W-7405-ENG-48 with funding support from NIH ES 09117-03.

**A new class of fluorescent dyes: Alexa Fluor 555 and Alexa Fluor 647 dyes are spectrally identical to Cy3 and Cy5 dyes, yet produce much brighter DNA probes.** *W.G. Cox, M.P. Beaudet, C. Ying, S. Yue, X. Chen, V.L. Singer, J.M. Beechem, R.P. Haugland.* Molecular Probes, Eugene, OR.

DNA labeling based on enzymatic incorporation of aminoallyl-dUTP (AA-dUTP), followed by reaction with a succinimidyl ester dye, is a reliable labeling method that eliminates the intrinsic dye-to-dye variability associated with incorporation of pairs of fluorophore-labeled nucleotides. Unlike fluorophore-labeled nucleotides, AA-dUTP is efficiently incorporated by DNA polymerase I and reverse transcriptase, and we have developed conditions under which the AA-dUTP:dTTP ratio used in the reaction mixture dictates the resulting degree of substitution in the labeled DNA molecule. In theory, the brightness of the labeled DNA should increase linearly with the number of primary amines introduced into the DNA. We found, however, that the brightness of labeled DNA varied in a non-linear manner when those amines were labeled with either Cy3 or Cy5 dyes. To solve this problem, we invented two new fluorophores, Alexa Fluor 555 and Alexa Fluor 647 dyes, which are spectrally similar to Cy3 and Cy5 dyes, yet do not show this anomalous behavior when attached to DNA. In characterizing this phenomenon, we found that DNA labeled with Cy3 and Cy5 dyes exhibited a change in absorbance spectra that occurred as a function of increased dye labeling. We found a progressive shift in the absorbance spectra of these labeled DNA molecules, from their initial long wavelength absorbance maxima to blue-shifted maxima that are completely non-fluorescent. This effect is especially pronounced for DNA labeled with Cy5 dye. The spectral shifts and associated reduction in fluorescence were eliminated by nuclease digestion of the labeled DNA, indicating that it is the interaction of the bound dyes with one another on the DNA that is problematic. We found that the use of Alexa Fluor 555 and Alexa Fluor 647 dyes greatly minimizes this effect, and yields labeled DNA that is much brighter than DNA labeled with Cy3 or Cy5 dyes at equivalent degrees of labeling. We are evaluating the relative performance of DNA labeled with Alexa Fluor dyes and Cy dyes, in a variety of applications, including microarrays and FISH.

**Novel features of genome organization revealed by single copy FISH.** *P.K. Rogan, P.M. Cazcarro, J.H.M. Knoll.* Med Genetics & Mol Medicine, Children's Mercy Hospital, Kansas City, MO.

Some highly-conserved paralogs are misaligned and reiterated intervals are underrepresented in the human genome reference sequence due to incorrect positioning of the respective contigs (Genome Res. 11:1005, 2001). While using repeat-masked sequences to design short 1.5-10 kb probes for FISH studies (scFISH; Genome Res. 11:1086, 2001) of clinically-significant chromosomal regions, we detected unexpected hybridization, consistent with misassembly and misclassification of single/low copy sequences. Probes from 3 of 24 regions studied to date, ie. the chromosome 21q22.2 Down Syndrome critical region (20, 30, and 39 kb upstream, respectively, of the DSCR4 gene, which themselves are embedded in a >100 kb segmental duplicon) and chromosomal regions disrupted in acute myelogenous leukemia-M4 (20 and 60 kb downstream of PM5 in 16p13; exon 6 and exon7-IVS7 of ATBF1 in 16q22), detected repetitive sequence families with localized, clustered distributions on the short arms of acrocentric chromosomes - in addition to their respective locations on chromosomes 21 and 16. These probe sequences are unrelated to the ribosomal RNA cistron, which colocalizes to the acrocentric short arms. Hybridization to the acrocentric short arms was eliminated either by suppression with C<sub>0</sub>t1 DNA or by high-stringency post-hybridization washes. We also found that members of a paralogous family of sequences near PM5, all of which were presumed distal and »1.3 mb telomeric of the 16p13 breakpoint in AML-M4, unexpectedly also mapped proximal to this breakpoint, based on their chromosomal locations in patients with this disorder. Sequence gaps are more dense and there is less redundant coverage in this region, consistent with lower reliability of ordering of these sequences. scFISH is likely to be useful in detecting other misassembled regions and other unrecognized repetitive sequence families. By differential labeling of flanking probes, scFISH may also be useful in verifying the existence and lengths of sequence gaps.

**Molecular cytogenetic characterization of a new case of partial trisomy 16q24.1-qter: literature review and phenotype-genotype correlations.** *S. Brisset<sup>1</sup>, G. Joly<sup>2</sup>, C. Ozilou<sup>1</sup>, J.M. Lapierre<sup>1</sup>, P. Gosset<sup>1</sup>, M. Le Lorc'h<sup>1</sup>, O. Raoul<sup>1</sup>, C. Turleau<sup>1</sup>, M. Vekemans<sup>1</sup>, S.P. Romana<sup>1</sup>.* 1) Department of Genetics, Hopital Necker-Enfants Malades, Paris, France; 2) Cytogenetics, CHU Charles Nicolle, Rouen, France.

We describe a 3 1/2-year-old girl with psychomotor and mental retardation, dysmorphic features including high forehead with bitemporal narrowing, a broad nasal bridge and a broadened nose, antimongoloid slant of palpebral fissures, abnormal ears, vertebral abnormalities, cardiac defect, genital hypoplasia and anorectal abnormalities. The karyotype of our patient (550 bands) was normal. Molecular cytogenetics techniques including comparative genomic hybridization (CGH) and FISH revealed that this girl was carrier of a de novo derivative chromosome 7 arising from a cryptic t(7;16)(p22.3;q24.1) translocation generating a trisomy 16q24.1-qter and a 7p22.3-pter deletion. FISH with a series of specific chromosome 7p and 16q probes permitted us to delineate the chromosome 7 breakpoint distal to YAC855A6 (WI-6692) and the chromosome 16 breakpoint between BAC457K7 (D42O53) and BAC442O1 (SGC30711). The comparison of the clinical features of our patient and those of 2 cases of pure terminal 7p deletion and 28 cases of trisomy 16q reported in the literature allowed us to establish the following phenotype-genotype correlations for trisomy of the long arm of chromosome 16: profound and severe mental retardation to band 16q12.1-13, intestinal abnormalities to 16q13 band, feet deformity (club feet) to 16q22 region, flexion/contractures of joints to 16q23 band, and dysmorphic features (high/prominent forehead, bitemporal narrowing, periorbital edema in the neonatal period), vertebral abnormalities, anorectal anomalies and low birth weight to band 16q24.

**Characterization of breakpoints in 67 Prader-Willi and Angelman syndrome deletion patients.** *M.C. Varela, C.P. Koiffmann.* Dept Biol, Univ Sao Paulo, Sao Paulo, Brazil.

Prader-Willi (PWS) and Angelman syndromes (AS) are distinct neurobehavioral disorders resulting from molecular defects in an imprinted cluster in chromosome 15q11-q13. PWS results from the loss of paternal gene expression, whereas AS is caused by loss of expression of a maternal gene (UBE3A). The chromosome segment 15q11-q13 is meiotically unstable with an unusual variety of cytogenetic rearrangements, including a common 4Mb deletion that occurs in 75% of PWS/AS patients. Here we present results on breakpoint analysis of 67 PWS/AS deletion patients (36 PWS; 31 AS), which strongly supports evidence indicating the presence of 3 specific breakpoint clusters in 15q (BP1-BP3). The patients were diagnosed by DNA methylation at SNURF-SNRPN exon 1, with the deletion extent detected by microsatellite analysis of 6 markers within and outside the critical region as well as 5 markers flanking BP1-3. No significant differences in the deletion breakpoint locations were observed between paternally or maternally derived deletions in PWS or AS, respectively. Of 63 patients analyzed for the proximal BP1-2 region markers D15SS541/D15S542, 54 were informative for at least one of two markers. Overall, 28% (15/54) were deleted for S541/542, indicating the deletion breakpoint being proximal to these loci (BP1). The other 72% (39/54) were heterozygous at S541/542, and of 34 patients were tested for D15SS543, all 12 informative cases showed deletion, while 22 uninformative cases had only one allele shared by both parents, indicating that the majority or all these cases had a proximal breakpoint at BP2. At the distal end of the deletions, all 36 informative cases were deleted for D15S1002 (none of the other 24 patients were heterozygous). In contrast, 0/39 informative cases were deleted for D15S1048. These results suggest a single major PWS/AS breakpoint cluster in the distal region of 15q11-q13 (BP3), between S1002 and S1048. In summary, we found only 3 breakpoint regions in PWS/AS deletion patients (BP1-BP3). Ongoing molecular studies will determine whether the PWS/AS breakpoints occur in HERC2- or other duplicons in these regions. Support: FAPESP, CEPID, PRONEX.



**Real-time PCR to detect a chromosome 1p36 deletion: A novel screening strategy for submicroscopic subtelomeric deletions.** *R.L. Touraine<sup>1</sup>, V. Adouard<sup>1</sup>, B. de Fréminville<sup>1</sup>, S. Pestre<sup>1</sup>, M. Till<sup>2</sup>, F. Prieur<sup>1</sup>, B. Lauras<sup>1</sup>.* 1) Genetique, CHU-Hopital Nord, Saint Etienne, France; 2) Genetique, CHU-Hopital Debrousse, Lyon, France.

Submicroscopic subtelomeric chromosome rearrangements are considered to be found in 5-10% of children with moderate to severe mental retardation and in 0.5-1% of children with mild mental retardation. Two principal techniques are used to detect these anomalies, namely FISH detection and microsatellite markers analysis. The utilization of the multitelomeric FISH analysis is limited by its cost. Microsatellites analysis is less informative and further requires the parents' DNA, making it expensive too. We decided to use a different approach based on locus quantification using real-time PCR. We first tested this method on a patient harboring a chromosome 1p36 deletion.

The patient was a young adult male with mild to moderate mental retardation and few dysmorphic features (large hands with short fifth fingers, small palpebral fissures, deep-set eyes, flat nasal bridge and pointed chin). De novo chromosome 1p36 was diagnosed on high resolution karyotyping and confirmed by FISH analysis. Real-time PCR was performed on a LightCycler (Roche) using either SYBR Green or hybridization probes. Loci for quantitative-PCR analysis were chosen in subtelomeric clones of chromosome 1pter and 1qter, according to the available databases.

We were able to show 1pter haploinsufficiency in our patient, together with a normal 1qter dosage. We found the opposite results in a control patient having a chromosome 1qter deletion.

This study established the feasibility of real-time PCR analysis for the screening of subtelomeric aneuploidies. We will discuss the respective advantages of SYBR Green and hybridization probes for the detection of haploinsufficiency with the LightCycler system. This new strategy represents a quicker and cheaper alternative to the multitelomeric FISH analysis.

**Cul1 and Skp1 complexes inactivation leads to defective chromosome segregation, genetic instability and neoplastic transformation.** *J. Liu, R. Piva, R. Chiarle, A. Podda, M. Pagano, G. Inghirami.* Pathology, NYU School of Medicine, 31st E.River Drive, NY, NY.

The ubiquitin dependent proteolysis involves a cascade of enzymatic reactions catalyzed by the E1 ubiquitin-activating enzyme, the E2 ubiquitin-conjugating enzymes, and the E3 ubiquitin-protein ligases. The substrate specificity of the ubiquitin pathway is conferred by the E3s. One such E3 is a multimeric protein complex designated SCF composed of Skp1, Cullin, F-box-containing proteins, and Rbx1/Roc1. The SCF complex promotes cell cycle transitions through degradation of multiple cell cycle regulators. To block SCF- and other Skp1-dependent activities, we generated Cul1 dominant-negative mutants (D-Cul1) capable of binding Skp1 but not Rbx1. As a proof of principle, D-Cul1 over-expression induced the accumulation of p27, b-catenin, cyclin E and p21, all targets for SCF-mediated ubiquitination. The expression of high levels of D-Cul1 resulted in hyperdiploid and multinucleated cells, with a concurrent number of centrosomes. Impaired chromosome segregation and chromosomal instability was strikingly seen in D-Cul1 expressing cells. Accordingly, FISH analysis confirmed that D-Cul1 cells underwent random and uneven chromosomal segregation resulting in cell growth defects. Over time, however, D-Cul1 expressing NIH-3T3 cells could overcome this proliferative block and generate tumors in nude mice. Transgenic (Tg) mice expressing D-Cul1 in the T-cell lineage showed hypocellularity in the thymus and peripheral lymphoid organs. Starting at week sixteen, D-Cul1 Tg mice displayed a high incidence (>80%) of CD4-CD8 double positive thymic lymphomas. Moreover, DCul1 Tg lymphomas and derived cell lines were aneuploid (85-90%) with widespread karyotype heterogeneity. Both depleted and neoplastic phenotypes in D-Cul1 Tg mice were partially rescued crossing D-Cul1 with Skp1 Tg mice, confirming the specificity of these findings and the crucial role of Skp1. Overall, our data demonstrate that Cul1 and possibly other Skp1 complexes are instrumental in the maintenance of chromosomal stability.

**Chromosome analysis of endometrial biopsy from a female IVF-candidate with 45X/46,XX mosaicism. H.E. Wyandt, S. Phansey-Chauhan, J.M. Milunsky.** Center for Human Genetics, Boston Univ Sch Medicine, Boston, MA.

A 26 year-old woman had a history of two IUI pregnancies that miscarried at 5 and 10 weeks. Her husband has a normal karyotype. The woman has 45,X in 43% of cells and 46,XX in 67% of cells from peripheral blood. One cell was 47,XXX. She has no major health problems. Menarche was at age 13 and she has had regular menses. She has normal hearing and vision and no known learning problems. Her weight is in the 10th centile, height in the 3rd centile and head circumference in the 90th centile. She has a prominent chin, posteriorly rotated ears, malocclusion, cubitus valgus, slightly hyperconvex nails, mild scoliosis and multiple nevi and freckles. Due to a planned IVF, an endometrial biopsy was obtained for hormonal assessment and concurrently submitted for chromosome studies. Three sites were cultured: site 1 had 45,X in 10/11 cells, site 2 in 9/20 cells and site 3 in 8/20 cells. Overall, 51% of cells were 45,X and 49% were 46,XX. No cells had 47,XXX. Two % of women with Turner syndrome (TS) reportedly have pregnancies (more likely, if they are mosaic or have a structurally abnormal X). A high rate of pregnancies miscarry or end in perinatal death, malformations and chromosome abnormalities. IVF is a proposed solution for TS patients, especially those who are mosaic. A few IVF pregnancies are reported, with a low rate of embryos transferred. Reasons cited are poor embryo quality and a high frequency of chromosome abnormalities in the embryos. We would like to suggest an additional possible impediment to successful IVF -namely problems in implantation due to mosaicism within the uterine lining. Because endometrium is replaced at each menses, it is conceivable the degree of mosaicism could vary in each pregnancy. To our knowledge, this idea is untested. Whether such mosaicism is significant in IVF success or not will require additional chromosome studies of pre-IVF endometrial biopsies and of pregnancy outcomes. The option of IVF is increasingly offered to high-risk infertility patients including those with TS. Chromosome study of an endometrial biopsy in TS patients is worth considering as a routine part of IVF work-up.

**The use of X-chromosome inactivation to determine the mechanism of UPD15.** *C. Fridman, C.P. Koiffmann.* Dept Biology, Univ Sao Paulo, Sao Paulo, Brazil.

Prader-Willi (PWS) and Angelman syndrome (AS) are neurobehavioral disorders resulting from the loss of expression of imprinted genes in the paternal or maternal chromosome 15, respectively, and can result from 15q11q13 deletion, uniparental disomy (UPD), imprinting mutation or UBE3A mutations in AS. Maternal (mat) or paternal (pat) UPD may arise through gamete complementation (GC), trisomic rescue (TR) or compensatory UPD (C). GC involves pat and mat meiotic errors, while TR and C involve a meiotic error and a postzygotic loss or gain of a chromosome, respectively. To identify the mechanism of UPD, X-inactivation (XCI) studies were used as an indirect test of trisomic rescue versus the other two mechanisms. Both mat and pat X chromosomes are active in pre-implantation embryos and random XCI occurs around the time of implantation in the fetal precursor cells. If matUPD arises from zygotic rescue, this chromosome loss can occur either early in development before XCI, which would be associated with random XCI, or shortly after XCI and in the latter case the presence of non-random XCI would be detected. Similarly, patUPD is a rare event and the embryonic timing of the gain of a chromosome 15 can be determined by the degree of XCI. We performed X-inactivation studies (DXS225) in 7 matUPD15 and 3 patUPD15 females, and defined non-random XCI as more than 90% inactivation of one X. Two cases (1PWS,1AS) were uninformative. Two patUPD15 showed random XCI; one was known to be due to meiotic II nondisjunction, but the hypothesis of TR could not be confirmed. Only one matUPD15 showed extremely skewed XCI (100%), while 5 cases showed random XCI (3 between 50-69%, one has 70% and one 80% skewing). In one case, trisomy 15 was detected by CVS testing followed by a normal karyotype in amniocentesis, and the matUPD15 was detected when the child was diagnosed with PWS, confirming the TR. The degree of skewing was 66:34, suggesting the loss of one chromosome 15 before XCI in the embryo proper. The data are consistent with most cases of matUPD15 and patUPD15 arising from an early TR or C mechanism, respectively, but a larger dataset is needed to accurately establish correlations between XCI and the mechanisms that generate UPD. Support: FAPESP, CEPID.

**Uniparental disomy: UK Collaborative Study.** *K.K. Smith<sup>1</sup>, T.A. Boyle<sup>2</sup>, D.L. Morgan<sup>1</sup>, C.A. Parkin<sup>3</sup>.* 1) Cytogenetics, Addenbrooke's NHS Trust, Cambridge, England; 2) Cytogenetics, Salisbury NHS Trust, Salisbury, England; 3) Cytogenetics, City Hospital NHS Trust, Nottingham, England.

An Association of Clinical Cytogeneticists Collaborative Study collected data from eighteen UK laboratories on prenatal and postnatal uniparental disomy (UPD) investigations.

**PRENATAL:** Fetal UPD 15 was confirmed in two cases of trisomy 15 confined to the placenta (CPM) and one case of apparent trisomy 15 pseudomosaicism in amniotic fluid. Fourteen cases with a supernumerary marker derived from 15 and 12 cases with a parent carrier of a Robertsonian translocation involving 15 were investigated and all showed biparental inheritance. Fifty prenatal cases where one parent carried a Robertsonian translocation involving chromosome 14 (24 cases maternal and 26 cases paternal) showed biparental inheritance. There were no cases of UPD7 or UPD6 detected. UPD16 was confirmed in two cases of trisomy 16 CPM.

**POSTNATAL:** UPD14 was confirmed in a female with precocious puberty, a phenotypically abnormal carrier of a balanced Robertsonian translocation involving 14 and in conjunction with trisomy 14 mosaicism. Four referrals for Silver-Russell syndrome/short stature showed UPD7 and one case of transient neonatal diabetes mellitus showed UPD6. UPD was found associated with either structural rearrangements or mosaicism for chromosomes 2, 6, 7 and 9.

**Towards an X inactivation profile of the mouse X chromosome.** *L.H. Chadwick, A. Cottle, H.F. Willard.* Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

While the majority of genes on the inactive X chromosome (Xi) are silenced by X inactivation, a subset of genes has been identified that escape from inactivation and are biallelically expressed. In a recent survey of 224 human X-linked transcripts, approximately 15% were found to escape inactivation in somatic cell hybrids (PNAS 96(25):14440-4). In contrast, the X inactivation profile of the mouse X chromosome is far less well defined. The few genes that have been tested are mainly restricted to the homologues of genes that escape inactivation in humans. Only five genes have been identified thus far that escape inactivation in mice. It is unclear whether this discrepancy between humans and mice reflects a difference in the regulation of X inactivation between these two species or merely a sampling bias. To address this question generally, we are conducting an ongoing survey of the X inactivation status of genes on the mouse X chromosome using RT-PCR and allele-specific restriction enzyme digestion. To generate informative polymorphisms for our assays, we used *M. domesticus* x *M. castaneus* F1 mice carrying Searles translocation (T16H), which show non-random inactivation of the normal X. Polymorphisms that distinguish these two alleles have been identified for twenty X-linked genes. Thus far, we have designed assays for seven of these genes; all seven showed monoallelic expression from the T16H chromosome, indicating that they are subject to inactivation. In addition, we are analyzing the relative levels of escape from inactivation of genes on the mouse X. Previous work has shown that *Smcx* expression from the Xi is roughly half that of the *Xa*, with some variability between individual mice. Using a quantitative allele-specific expression assay, we have confirmed these data and have determined the relative level of escape for *Utx*. On average, we have found *Utx* expression from the Xi to be nearly 80% of the active X expression, significantly higher than what has been demonstrated for *Smcx*. These data indicate that the local chromatin environment may vary across the Xi, determining the likelihood of escape from inactivation and/or the degree of silencing.

**Variability in DNA methylation assays of X chromosome inactivation (XCI).** *C.L. Beever, R.H. Jiang, C.J. Brown, W.P. Robinson.* Department of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada.

Skewed XCI is currently used as a biological marker for clonality and carrier status of certain X-linked diseases as well as for research into various biological processes and disorders. With the broad use of XCI assays, unbiased measurement of the degree of XCI skewing is necessary. XCI assays generally involve amplification by PCR of an X-linked locus which is both differentially methylated, allowing distinction of the inactive and active X via digestion with a methylation-sensitive enzyme (HpaII), and polymorphic, allowing discrimination of alleles of the locus. To test the reproducibility of such assays, we examined the effect of the following on XCI results: 1) locus (androgen receptor (AR) vs. FMR-1); 2) the use of a secondary cutter (RsaI) during digestion with HpaII; and 3) quantification methods (densitometry of silver-stained gels vs. automated fluorescent analysis (AFA) using an ABI 310). The mean difference in percent inactivation of one allele in 12 samples tested with both AR and FMR-1 was  $10.7\% \pm 2.0\%$  (mean  $\pm$  SE) with a range of 0-26. One case was 99% skewed with FMR-1 and 75% skewed with AR. Further analysis of this sample with a third locus, DXS6673E, was consistent with the AR results, suggesting additional influences upon FMR-1 methylation make this assay less reliable. Using the AR locus, the mean difference among 77 samples assayed with and without a secondary cutter was  $6.4\% \pm 0.7\%$  with a range of 0 to 35. Overall, a slight tendency to greater skewing was seen when a secondary cutter was used, perhaps reflecting reduced secondary structure formation that could impede digestion by HpaII or amplification. Although results from densitometry and AFA were highly correlated for 18 samples tested by both methods, within sample reproducibility was higher with AFA. In conclusion, we recommend using the AR locus with a secondary cutter and AFA. With this methodology we observed a mean difference in 45 replicated samples of only  $2.9\% \pm 0.7\%$ . Additional studies, including comparisons to expression based assays, would validate the accuracy of measuring XCI skewing based on DNA methylation at a single locus.

**Identification of autoimmune sera that target the Barr body.** *W.H. Brooks<sup>1</sup>, B. Hong<sup>1</sup>, M. Satoh<sup>2</sup>, W.H. Reeves<sup>2</sup>, P. Reeves<sup>3</sup>, B. Panning<sup>3</sup>, T.P. Yang<sup>1</sup>.* 1) Dept. of Biochem. & Mol. Biol., Univ. of Florida, Gainesville, FL; 2) Dept. of Rheumat. & Clin. Immunol., Univ. of Florida, Gainesville, FL; 3) Dept. of Biochem. & Biophys., Univ. of California at San Francisco, San Francisco, CA.

Early in mammalian embryogenesis, one of the two X chromosomes in female somatic cells is transcriptionally inactivated to achieve dosage compensation of X-linked genes in males and females. The resulting inactive X chromosome appears as a dense, peripheral, heterochromatic structure in the nucleus, termed the Barr body. Little is known about the structure and molecular composition of the Barr body. Because sera from autoimmune patients commonly contain antibodies against a variety of nuclear structures, we used indirect immunofluorescence to screen sera from 440 autoimmune patients for antibodies against the Barr body as an initial step to investigate the composition of the Barr body. Autoimmune diseases represented by the patients included systemic lupus erythematosus (SLE), scleroderma, mixed connective tissue disease, discoid lupus and others. Each serum was applied to XY male and XXX female human fibroblasts grown on slides, and examined for preferential immunostaining of the Barr body. Two sera were identified that stained a distinct nuclear structure consistent in size, location, and number with the Barr body. Immunostaining of female fibroblasts by the first serum demonstrated co-localization with *XIST* RNA. This serum also stained all human metaphase chromosomes and a nuclear structure consistent with the inactive X in female mouse fibroblasts. Staining of metaphase chromosomes did not show preferential staining of centromeres, indicating the antigen(s) is unlikely to be a general heterochromatin-associated protein. Immunostaining of cells from E7.5 mouse embryos did not show co-localization with *Xist* RNA, suggesting the antigen(s) is unlikely to be associated with initiation of X inactivation. Analysis of the second serum, from a female SLE patient, is currently in progress. These sera provide a novel strategy for investigating the composition and dynamics of the Barr body and demonstrate that one or more components of the Barr body are autoantigens in some autoimmune diseases.



**Skewed X-inactivation in extraembryonic tissues and early embryonic lethality in humans.** *V.N. Evdokimova*<sup>1, 2</sup>, *S.A. Nazarenko*<sup>2</sup>. 1) Dept Biological Sci, Florida Intl Univ, Miami, FL; 2) Institute of Medical Genetics, RAMS, Tomsk, Russia.

Genetic inactivation of one X-chromosome appears in early embryonic development of mammals and leads to a monoallelic expression of X-linked genes in the somatic cells. Repression of one of X-chromosomes inherited from either mother or father in 100% of the cells can create uniparental expression pattern even in the normal 46,XX females without uniparental disomy by X. Most mammal species have imprinted paternal X-inactivation in the extraembryonic cell lines. In terms of evolution the distribution of imprinted paternal X-inactivation tends to decrease from whole organism (in marsupials) to the restricted cell lines (in mice). The patterns of preferential X-inactivation in humans is not completely clear. The goal of present study was to show the patterns of X-inactivation in extraembryonic tissues in spontaneous and induced abortions during the first trimester of development with normal 46,XX karyotype. The DNA samples that were extracted from extraembryonic tissues dissected from spontaneous (52) and induced (37) abortuses with normal female karyotype - 46,XX and DNA samples from their parents were investigated. The karyotypes were obtained by cytogenetic analysis and verified by PCR analysis with a set of chromosome-specific STR-markers. Skewed X-inactivation was determined using the AR model. PCR amplification was performed with TET-labeled primers. The PCR products were separated by automated capillary electrophoresis in an ABI PRISM 310 Genetic Analyzer. The size and ratio of AR alleles are calculated automatically using GeneScan Software (Perkin Elmer). Two types of X-inactivation were found in spontaneous abortions (SA) and induced abortions (IA): stochastic and skewed. Stochastic X-inactivation was in 53.2% of SA and in 40.0% of IA. However, the comparative analysis of the distribution of X-inactivation patterns in the different morphological groups of the spontaneous abortions (blind ovums, missed abortuses, spontaneous abortuses) indicated the tendency to increasing asymmetrical X-inactivation depends on the severity of damage in the embryonic development.

**Mouse *Prophet of Pit1 (Prop1)* gene knockout.** I. Nasonkin, S. Camper. Human Genetics, University of Michigan, Ann Arbor, MI.

*Prophet of Pit1 (Prop1)* is one of several homeodomain transcription factors that are required for the development of the hormone producing cell types of the anterior pituitary gland. During embryogenesis *Prop1* regulates the expression of other homeobox genes, extinguishing *Hesx1* and activating *Pit1*. Ames dwarf mice have a Ser83Pro mutation in the homeodomain of *Prop1* that causes a partial loss of function and leads to profound pituitary hypoplasia. The *Pit1* lineage fails to develop, resulting in GH, TSH and PRL deficiency. Gonadotropins are also reduced, but corticotropes appear unaffected. *Prop1* is usually extinguished by embryonic day 16. Persistent expression of *Prop1* interferes with gonadotrope differentiation and thyrotrope function, and increases the incidence of pituitary adenomas. Thus, regulation of *Prop1* expression is important for normal pituitary development and function. Eleven different mutations in *PROP1* have been described in human patients with Combined Pituitary Hormone Deficiency (CPHD), with an A301G302 deletion being the most common. The age of onset, effect on pituitary size and number of hormone deficiencies is variable, but patients generally lack GH, TSH, PRL, LH and FSH. ACTH deficiency occurs rarely. There is no obvious genotype-phenotype correlation, suggesting that other genes may influence the severity of the endocrine deficiency. In order to understand the role of *PROP1* in pituitary development we created a null allele by gene targeting. Mice homozygous for the *Prop1* null allele exhibit differentiation of corticotropes and gonadotropes, but the hypoplasia of the anterior lobe is more severe than in the Ames dwarf. In addition, 50% of the homozygous null mice die before weaning, while Ames dwarf mice are viable. This null allele will be a valuable tool for dissecting the role of *Prop1* in corticotrope and gonadotrope function.

**AP-2a Transcription Factor Mutation Demonstrates the Power of ENU.** *N. Ahituv*<sup>1</sup>, *A. Erven*<sup>2</sup>, *H. Fuchs*<sup>3</sup>, *R. Balling*<sup>3</sup>, *M. Hrabe de Angelis*<sup>3</sup>, *K.P. Steel*<sup>2</sup>, *K.B. Avraham*<sup>1</sup>. 1) Department of Human Genetics & Molecular Medicine, Sackler School of Medicine, Tel-Aviv University, Israel; 2) MRC Institute of Hearing Research, University Park, Nottingham, UK; 3) GSF Center of Environment & Health Institute of Experimental Genetics, Neuherberg, Germany.

Mice serve a vital role as a model for studying human genetic hearing loss. Due to the similarities between mouse and human genomes, auditory and vestibular function and morphology, mice are being used both for isolating genes and characterizing the inner ear. Using N-ethyl-N-nitrosourea (ENU), a chemical mutagen, we are generating deaf and vestibular mouse models to search and study the function of different genes and proteins in the auditory and vestibular system. One of the advantages of using ENU is that it mutates genes randomly, causing single nucleotide changes, and provides the possibility of learning more about protein site-specific functions. Several dominant ENU induced deaf and vestibular malfunction mouse mutants have been characterized on a phenotypic level, and were mapped to chromosomal regions.

Doarad (*Dor*), a dominant ENU-derived mutant, has a misshapen incus and mildly-abnormal malleus and stapes, leading to deafness. *Dor* was found to map to a 5.5 cM region on mouse chromosome 13. This region contains AP-2a, a member of a family of transcription factors found to function as regulators in a large spectrum of important biological functions, including cell-cycle control, apoptosis and vertebrate development. AP-2a homozygous knockout mice exhibit failure of cranial neural-tube closure at embryonic day 9, which leads to skeletal defects and anencephaly; mice die perinatally due to severe congenital defects. Our candidate gene analysis of *Dor* revealed a C®T missense mutation in AP-2a that changes a highly conserved proline, located in the PY motif of the activation domain, to leucine. No live homozygotes were detected in a total of 4 litters (n=18), suggesting that the *Dor* mutation in a homozygous state is lethal. This is the first report of a mutation in a gene specifically causing middle ear abnormalities, leading to conductive hearing loss.

**Genetic Modifier of Mouse Eya1BOR Mutation**<sup>BOR</sup>. *D.A. Gold*<sup>1,2</sup>, *D. Concepcion*<sup>2</sup>, *X. Wang*<sup>2</sup>, *E.M. Keithley*<sup>2</sup>, *A.F. Ryan*<sup>1,2</sup>, *B.A. Hamilton*<sup>1,2</sup>. 1) Biomedical Sciences, UC San Diego, San Diego, CA; 2) School of Medicine, UC San Diego, San Diego, CA.

Mutations in the EYA1 gene cause dominant branchio-oto-renal syndrome (BOR) in humans. A spontaneous mouse mutation, Eya1<sup>BOR</sup>, causes similar phenotypes in inner ear and kidneys with recessive inheritance. Each Eya1<sup>BOR</sup> allele expresses approximately 50% normal RNA levels due to insertion of an endogenous retrovirus. We have observed strong variation in Eya1<sup>BOR</sup> homozygous phenotypes on a mixed genetic background. Using histology, auditory-evoked brainstem responses (ABR) and northern blot analysis, we see variation of the Eya1<sup>BOR</sup> phenotype at morphological, physiological, and molecular levels. Our preliminary data suggests that this effect maps near the Mvb1 locus, which has similar effects on the mouse vibrator mutation.

**Definition of co-ordinately regulated gene clusters in muscle regeneration.** *P. Zhao*<sup>1,2</sup>, *Y-W. Chen*<sup>1</sup>, *D. Dressman*<sup>1</sup>, *E.P. Hoffman*<sup>1</sup>. 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Genetics Program, George Washington University, Washington, DC.

Muscle regeneration is important in inherited muscle disease, and slowly fails in the progressive dystrophies. Muscle regeneration is important to development geneticists as it is believed to recapitulate embryonic muscle development. Here, we report the first use of expression profiling in tissue regeneration *in vivo*. We present the identification of co-ordinately regulated gene clusters in staged skeletal muscle degeneration/regeneration induced by cardiotoxin. Mouse gastronemius muscles at 6 time points (duplicate muscles at 0, 12h, 1 day, 2 day, 4 day and 10 day) after cardiotoxin or PBS injection were profiled using Affymetrix murine genome U74A chips (10,000 genes/ESTs). Gene expression patterns were temporally clustered using GeneSpring. We nucleated gene clusters using known muscle regeneration markers (myogenin, MyoD, myf5, M-cadherin, N-cadherin, acetylcholine receptors). We identified a large number of novel genes involved in muscle regeneration, including transcription factors (Sox11, Slug), signaling pathway proteins (Calpain6, WISP-1, Peg3, Ulip), and extracellular matrix proteins (matrilin-2, osteoglycin, Col15a1, Col8a1). We further tested the hypothesis that temporal clustering is able to lead to identification of novel downstream targets of the myogenic regulatory factor, MyoD. We used a known MyoD downstream target to nucleate a temporal cluster. We found MyoD binding site (E-box) in the putative promoter region of several novel cluster members. We then verified some of these as regulated by MyoD by promoter gel shift assay. Our results show that genome-wide expression profiling method can be utilized in an *in vivo* model of muscle regeneration. Moreover, we show that *in vivo* temporal clustering is an effective approach to identify novel downstream targets of myogenic transcription factors.

**REDUCTED LEVEL OF DYTROPHIN Dp71 INHIBITS NEURONAL DIFFERENTIATION OF PC12 CELLS.**

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Duchenne muscular dystrophy (DMD) is caused by mutations in the DMD gene. DMD gene produces at least seven different dystrophin isoforms and the smallest one, Dp71 has a unique N-terminus of seven amino acids but shares the cystein-rich and C-terminal domains with all other dystrophins. Dp71 is the most abundant dystrophin in the nervous system and its expression has been correlated with stages of terminal neuronal differentiation. Likewise, it is thought the Dp71 is involved in the DMD-associated mental retardation present in 30% of the patients. In order to ascertain the function of the Dp71 in neuronal cells we have adopted the PC12 cells as a study model. These cells respond to the nerve growth factor and differentiate into cells morphologically and biochemically similar to sympathetic neurons. Previously we found that two different splicing isoforms of Dp71 are expressed in PC12 cells, and interestingly, splicing isoform, which lacks exon 78, augments, its expression during neuronal differentiation. If Dp71 has an important role in neuronal differentiation, it could be expected that its absence affect the mentioned cellular process. Therefore, we decide to inhibit the expression to Dp71 in PC12 cells by antisense treatment. Vectors, expressing sense and antisense Dp71 RNAs, were constructed and stable transfectans clones were isolated by neomycin resistance. To select clones with reduced levels of Dp71, western blot and immunofluorescence assays, using anti-Dp71 antibodies, we performed. Selected Dp71-deficient clones present a marked inhibition of neurite outgrowth, after NGFA treatment. Confocal microscopy analysis of proteins belonging to the dystrophin associated proteins complex showed that these proteins are affected by the reduction of Dp71. Results suggest that Dp71 and its associated proteins play an important role during neuronal differentiation.

**Lateralized expression of sex chromosome genes in a gynandromorphic finch does not predict masculine brain phenotype.** *R.J. Agate*<sup>1</sup>, *S. Mann*<sup>2</sup>, *C. Schanen*<sup>2</sup>, *A. Palotie*<sup>2, 3</sup>, *A. Arnold*<sup>1</sup>. 1) Physiological Science, UCLA, Los Angeles, CA; 2) Human Genetics, UCLA, Los Angeles, CA; 3) Pathology, UCLA, Los Angeles, CA.

A prominent question regarding sexual differentiation of the neural song system in zebra finches is whether this difference is induced by sex-specific action of gonadal steroid hormones, by direct sex-specific action of sex chromosome genes expressed in the brain, or by some combination of these mechanisms. We have analyzed the brain/behavior of a zebra finch bilateral gynandromorph to shed light on this question. The plumage color was male on the right, female on the left. Its behavior was completely masculine in that it sang a masculine song, courted females successfully and copulated as a male. The amount of DNA per blood cell was in the diploid range. The gonads were lateralized, with a testis on the right and mostly ovarian tissue on the left. The neural circuit for song was masculine on both sides.

In birds males are the homogametic sex (ZZ) and females the heterogametic sex (ZW). The W chromosome is small and heterochromatic like the Y chromosome in mammals. In this bird, W-specific genomic DNA encoding CHDW was found predominantly on the left side in brain and feather pulp by PCR. The mRNA encoding the W-linked gene ASW, expressed only in genetically female brains, was expressed at high levels on the left side of the brain but was low or absent on the right. These results indicate that the bilateral masculine neural phenotype does not correlate with expression/distribution of two W-chromosome genes, compatible with a theory of hormonal induction of sex differences in brain. A direct role for genetic factors cannot be completely excluded by the available data because, for example, side-specific rearrangement of the sex chromosomes could have resulted in the lateralized expression of some genes regulating sexual differentiation of gonads and plumage but not brain. Supported by: NIH DC00217

**HOX-B1 allelic variants in hindbrain malformations.** *V. Capra<sup>1</sup>, A. Moroni<sup>1</sup>, P. De Marco<sup>1</sup>, E. Merello<sup>1</sup>, L. Arata<sup>1</sup>, M. Crippa<sup>2</sup>, F. Blasi<sup>2</sup>, A. Cama<sup>1</sup>.* 1) Lab Servizio Di Neurochirurgia, Inst Scientifico G Gaslini, Genova, Italy; 2) Laboratorio di Genetica molecolare, DIBIT-HSR, Milan-Italy.

Hindbrain malformations are a group of congenital developmental anomalies involving cerebellum, brain-stem, 4th ventricle, and cervical spinal cord. Chiari complex is the most frequent form and is characterized by caudal cerebellar herniation, sometimes associated with lower brainstem dysmorphism, skull base and vertebral anomalies. In vertebral hindbrain, generation of regional diversity is achieved through a segmentational process that leads to the formation of 7 rhombomeres. Hox genes display a key role in controlling, regulating neuronal migration and maintaining cellular segmental identity. Hoxb-1 gene is the first, in addition to Hoxa-1 gene, to be activated in CNS; moreover its expression domain is selectively restricted to rhombomere 4 and neural crest cells that from r4 migrate in the second branchial arch. Mutant mouse analysis and expression studies has shown that Hoxb1 gene is the first, in addition to Hoxa1, to be activated in CNS and it is the only one to show a regionally restricted expression domain (r4). We performed, by means of SSCP analysis, a mutational screening of the homologous HOX-B1 gene in 64 patients and 198 control individuals. Sequencing of abnormal SSCP conformomers revealed the existence of three allelic variants characterized by the presence of several in cis associated mutations, at the NH2 terminal region of the gene. The a1 haplotype, distributed according the Hardy-Weinberg equilibrium, is due to a 9-bp tandem duplication (CCCACAGCG) at position 80 associated to two synonymous transitions (C237T and G450A) and one missense mutation (A309T). The a1A allele, that differs from a1 for the absence of the A309T and the G450A was identified in 2 unrelated patients, while the a2 haplotype, showing three silent substitutions (G114A, C213T and G246A) and the C167T missense mutation, was isolated in three additional patients. Since a1A and a2 haplotypes were present in five healthy parents, but not in the control population, we can't directly correlate these mutations to the disorder but they could be predisposing factors to this complex malformation.



**Down syndrome cell adhesion molecule (DSCAM): a role in spinal cord differentiation and cortical plasticity?**

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Molecules involved in axon guidance play central roles in the development and plasticity of the human central and peripheral nervous systems (CNS and PNS) through their effects on neurite outgrowth, differentiation and synaptic transmission. In Down syndrome, brain characteristics include defective cortical lamination, cerebral and cerebellar hypoplasia and abnormalities of the dendritic tree. We have previously described DSCAM (Down Syndrome Cell Adhesion Molecule), an axon guidance molecule that is expressed in CNS and PNS neurons from the time of neurite differentiation and which maps to chromosome 21q22 in a region associated with DS mental retardation. We now report evidence supporting a role for DSCAM in cortical plasticity and in spinal cord differentiation. To evaluate the role of DSCAM in morphogenetic and cognitive processes, we examined DSCAM expression in the adult mouse brain using tissue in situ hybridisation (TISH) at three, six, nine and twenty-one months of age. The results indicate that DSCAM is widely expressed in the mouse brain throughout adult life with highest levels in the hippocampus, the midlayers of the cortex, the olfactory bulb and the purkinje cells of the cerebellum. These are regions which display continued neurogenesis and synaptic plasticity, supporting a role for DSCAM in prenatal CNS defects and learning and memory in DS. We further report the identification of a novel homolog, DSCAM2, whose predicted protein product has the same domain structure and over 55% amino acid identity to DSCAM (hereafter DSCAM1). Comparisons of DSCAM1 and DSCAM2 expression using TISH demonstrate complementary patterns of expression in the spinal cord during fetal development and distinct patterns in the adult mouse brain. We propose that DSCAM1 and DSCAM2 play different roles both in the developing CNS and in adult synaptic plasticity, and that their over- or underexpression contributes to the neurocognitive defects of DS.

**Sim1 haploinsufficiency causes hyperphagia, obesity and reduction of the paraventricular nucleus of the hypothalamus.** *J.L. Michaud<sup>1</sup>, F. Boucher<sup>1</sup>, A. Melnyk<sup>2</sup>, F. Gauthier<sup>1</sup>, E. Goshu<sup>3</sup>, E. Levy<sup>1</sup>, G.A. Mitchell<sup>1</sup>, J. Himms-Hagen<sup>2</sup>, C.M. Fan<sup>3</sup>.* 1) Research Center, Hopital Sainte-Justine, Montreal, QC; 2) Department of Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, ON; 3) Department of Embryology, Carnegie Institution of Washington, Baltimore, MD.

The bHLH-PAS transcription factor SIM1 is required for the development of the paraventricular nucleus (PVN) of the hypothalamus. Mice homozygous for a null allele of *Sim1* (*Sim1*<sup>-</sup>) lack a PVN and die perinatally. In contrast, we show that *Sim1*<sup>+/-</sup> mice are viable but display increased weight gain beginning at 4 weeks of age. By 24 weeks of age, the weight of *Sim1*<sup>+/-</sup> males and females is 33% and 40% higher than that of controls, respectively. This obesity is associated with increased linear growth, white fat hyperplasia and brown fat hypertrophy. *Sim1*<sup>+/-</sup> mice are normoglycemic but develop hyperinsulinemia and hyperleptinemia with increasing weight. Hyperphagia is observed in these mice beginning at 4 weeks of age, before the onset of obesity, and is sustained as 35-week-old *Sim1*<sup>+/-</sup> males and females eat 39% and 30% more than controls, respectively. In contrast to other mouse models of early-onset obesity, such as *McR4*<sup>+/-</sup> and *ob/ob* mice, oxygen consumption and pair-feeding experiments showed that their energy expenditure is not decreased. Quantitative histological comparison with normal littermates revealed that the PVN of *Sim1*<sup>+/-</sup> mice is smaller (+/+ : 8.6x10<sup>5</sup> mM + 2.7x10<sup>4</sup>; +/- : 7.1x10<sup>5</sup> mM + 2.3x10<sup>4</sup>, p<0.002) and contains on average 31% fewer cells as shown by nuclear profile counts (+/+ : 5823 + 259; 4431 + 168; p<0.005) and measurements of area occupied by nuclei (+/+ : 2.8x10<sup>5</sup> mM + 1.0x10<sup>3</sup>; +/- : 2.1x10<sup>5</sup> mM + 7.4x10<sup>3</sup>; p<0.005). Marker analysis did not show a selective loss of any identifiable major cell type. Since acquired lesions in the PVN also induce increased appetite without a decrease in energy expenditure, we propose that abnormalities of PVN development cause the isolated hyperphagia of *Sim1*<sup>+/-</sup> mice. Severe obesity has been described in a patient with a balanced translocation disrupting SIM1. Pathways controlling the development of the PVN thus have the potential to cause obesity in both mice and humans.

**Expression of sex chromosome genes in mouse brain.** *J. Xu*<sup>1</sup>, *P. Burgoyne*<sup>2</sup>, *A.P. Arnold*<sup>1</sup>. 1) Dept. of Physiological Science, UCLA, Los Angeles, CA; 2) Div. Developmental Genetics, MRC National Inst. For Medical Research, Mill Hill, London UK.

A major question is whether genes encoded on the sex chromosomes act directly in non-gonadal tissues to cause sex differences in development or function, or whether all sex differences in somatic tissues are induced by gonadal secretions. We use the brain, a sexually dimorphic tissue, as a model system to explore this question of sex-linked genes vs. hormones. The sex chromosomes could directly influence sexual differentiation via several mechanisms including sex differences in the expression of genes on the non-recombining portion Y (NRY) chromosome or because of the presence of sex differences in dose of X genes. Based upon the following results, both factors may attribute to sex differences of the brain.

We performed RT-PCR analysis to determine the neural expression of mouse NRY genes at three developmental stages, 13.5 days post coitus (13.5 dpc), postnatal day 1 (P1) and adult. Ubiquitously expressed Y genes *Smcy*, *Eif2s3y*, *Uty* and *Dby* were detected in brain at all three ages examined. Y genes *Ube1y*, *Usp9y* and *Ssty* have been thought to be expressed only in testis, but we detected authentic transcripts from these genes in brain at one or more ages. *Zfy* and *Rbmy* were not detected in the brain at any age.

We also detected transcripts of the X homologues *Smcx*, *Eif2s3x*, *Utx*, *Dbx*, *Ube1x* and *Usp9x* in the brains of both sexes at all three ages. These X-linked homologues of the NRY genes were not always expressed in parallel with the NRY genes, suggesting differences in regulation of the two forms. In adulthood, Northern blot analysis indicated that the expression of all six of these X genes is higher in adult female brain than adult male brain, irrespective of their X-inactivation status. The higher expression in female brain is not a general feature of all X genes, because no sex difference was found in the expression of *Hprt*.

**Insights into the regulation of hedgehog signalling from subcellular localisation studies.** *C.A Wicking<sup>1</sup>, T. Evans<sup>1</sup>, B. Wainwright<sup>1</sup>, R. Parton<sup>1,2</sup>.* 1) Inst for Molecular Bioscience, Univ Queensland, Brisbane, Australia; 2) Department of Physiology and Pharmacology, Univ of Queensland, Brisbane, Australia.

Dysregulation of the hedgehog signalling pathway is the major determinant of the most common form of cancer, basal cell carcinoma (BCC). In addition this highly conserved pathway is pivotal to the normal development of a mammalian embryo, with alterations in several key members resulting in developmental disorders. In particular, mutations in the hedgehog receptor molecule patched are responsible for both inherited and sporadic forms of BCC, as well as the developmental anomalies associated with the autosomal dominant disorder Gorlin syndrome. The current model of hedgehog signalling predicts that the patched protein acts as a receptor for the hedgehog ligand in a preformed complex with the smoothed molecule. Despite this role for patched in the cell surface reception of hedgehog, several studies have reported an absence of protein at the cell surface at any given time. We sought to clarify the subcellular localisation of both patched and smoothed in an attempt to more fully understand the cellular processes involved in hedgehog signalling. Using a combination of immunofluorescent and immunoelectron microscopy we show that patched localises primarily to the endocytic pathway of mammalian cells, with very little evidence of staining at the cell surface. Most notably we show that patched exists in recycling endosomes, and to a lesser extent in endocytic carrier vesicles and late endosomes. In contrast, transiently transfected smoothed was shown by immunoelectron microscopy to reside in the endoplasmic reticulum. We also show that patched and smoothed in transfected cells associate with detergent resistant membranes or lipid rafts which are rich in cholesterol. This finding is significant since several factors have implicated a major role for cholesterol in hedgehog signalling and in development in general. Combined with recent data from the *Drosophila* system, the results from this work support a shift in how we view the cellular and molecular processes involved in the regulation of hedgehog signalling.

**Hypoglycemia and abnormal metabolism associated with failure-to-thrive of neonatal Prader-Willi syndrome**

**mice.** *J.L. Knepper<sup>1</sup>, R.S. Ahima<sup>1</sup>, H.R. Patel<sup>1</sup>, T. Ohta<sup>1</sup>, E.M. Rinchik<sup>2</sup>, S. Bogdanovich<sup>1</sup>, T.S. Khurana<sup>1</sup>, R.D. Nicholls<sup>1</sup>.* 1) University of Pennsylvania, Philadelphia, PA; 2) Oak Ridge National Laboratory, Oak Ridge, TN.

Prader-Willi syndrome (PWS) infants have hypotonia and failure-to-thrive, with onset of hyperphagia and obesity after ~2 years of age, abnormal fat and muscle distribution, short stature with growth hormone deficiency, hypogonadism, and abnormal behavior. The underlying genetic defect is a loss of paternal imprinted expression in chromosome 15q11-q13, with PWS commonly ascribed to a hypothalamic deficiency. PWS mouse models have failure-to-thrive and hypotonia, and survive only ~1 wk. Analyses of body weight in postnatal ~1 day old (P1) PWS vs. wildtype (WT) mice showed growth retardation in the former (1.45±0.10 vs. 1.79±0.17 g., n=17-22; p<0.0001), with similar results at P3.5 (1.3±0.10 vs. 2.69±0.24 g., n=4-8; p<0.0001). At P5, PWS pups are severely hypoglycemic (22±1.8 vs. WT, 129 mg/dl; n=4, p<0.0001), and had low insulin levels (0.26 vs. 2.19 ng/ml). Oxygen consumption (VO<sub>2</sub>) and heat, measured by indirect calorimetry (ambient temperature 22°C), were markedly reduced in PWS (768 ml/kg/hr and 0.02 kcal/h) vs. WT mice (3981 and 0.234 kcal; p<0.0001). Carcass analysis showed that PWS and WT pups did not differ in water (68% vs. 63%, p=0.47) or protein (32% vs. 29%, p=0.4) content, but differed significantly in triglyceride content (4.3% vs. 0%). Our data suggest that neonatal mortality in PWS is likely as a result of severe hypoglycemia and impaired thermogenesis. Studies are underway to further characterize feeding behavior, plasma hormones, muscle and liver metabolism and gene expression in PWS and WT mice, as well as rare escaper PWS mice that survive into adulthood.

**Paired interstitial duplications and deletions: a novel cause of ocular developmental abnormalities and glaucoma.**

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Mutations in the forkhead transcription factor gene *FOXC1* cause a range of ocular developmental abnormalities associated with the development of glaucoma. However mutations are not present in all pedigrees which map to the vicinity of the 6p25 forkhead gene cluster. Our identification of comparably sized interstitial duplications in two families and an interstitial deletion (encompassing *FOXC1* and neither *FOXF2/FOXQ1*) in a Rieger syndrome pedigree, supports the concept that a common mechanism, presumed to be homologous recombination, is responsible for these 6p25 rearrangements and ocular developmental abnormalities. These findings represent the first example of both interstitial duplications and deletions causing a developmental phenotype attributable to altered transcription factor dosage and extend the limited number of disorders in which pairs of interstitial cytogenetic abnormalities have been observed. The interstitial duplications cause a contiguous gene syndrome, suggest that such cytogenetic rearrangements result in more extreme phenotypes than *FOXC1* coding mutations and provide additional evidence for the pathogenicity of altered *FOXC1* dosage.

**HOXD13 GENE ANALYSIS IN SUBJECTS WITH COMPLEX DIGITAL ANOMALIES.** *A. Perez-Cabrera, J.C. Zenteno, S. Kofman-Alfaro.* Department of Genetics, Hospital General de Mexico, Mexico City, DF, Mexico.

Homeodomain-containing homeotic genes (Hox) have been remarkably well conserved during animal evolution and are essential for the appropriate organization of the body plan during development. Several studies on the expression patterns and mutant phenotypes of these genes have indicated that the proper development of digits (number and size) in tetrapods requires the activity of several genes of the HOXA and HOXD complexes, particularly those belonging to posterior (5' located) groups 11, 12 and 13. disruption of the Hoxd13 gene in mice produced a limb malformation pattern characterized by reduction in the length of some bony elements, loss of phalanges, bone fusions and the presence of an extra element. In addition, synpolydactyly, a condition consisting of 3/4 syndactyly in the hands, 4/5 syndactyly in the feet and digit duplication in the syndactylous web, is due to expansions of an amino-terminal polyalanine tract or partial deletions in HOXD13. As HOXD13 might be implicated in other congenital digital anomalies, we search for molecular alterations of this gene in individuals with complex digital malformations. We included in the study a four member family with triphalangeal thumb-brachyectrodactyly syndrome and two unrelated subjects with a digital malformative pattern characterized by polydactyly, syndactyly and brachydactyly in hands and feet. We perform PCR amplification of the complete coding region of HOXD13 and automated sequence analysis of PCR products. After sequencing the two exons and the intron/exon boundaries of HOXD13 we were not able to detect any deleterious mutation in these subjects. We identified a previously undescribed silent polymorphism at nucleotide position 291 in exon 1 (GCA to GCG without changing the encoded alanine). Our data excludes that mutations in HOXD13 are a common cause of complex digital anomalies.

**Relative Difference Analysis of Cell Lines Stably Expressing the C-terminal Isoforms of the Receptor Tyrosine Kinase RET.** *L. Mulligan, S. Myers.* Dept Pathology, Queen's Univ, Kingston, ON, Canada.

The receptor tyrosine kinase RET is essential for early development of the kidney and the enteric nervous system. A number of splice variants of the *RET* transcript have been identified that result in proteins varying in the number of C-terminal amino acids, specifically encoding 9, 43 or 51 unique amino acids (RET9, RET43, RET51). In vitro studies have indicated that the RET9 isoform may contribute to cell growth while the RET51 isoform may be involved in both cell growth and cell differentiation. In agreement with this, we have previously reported differential expression of these isoforms of RET in the developing human kidney. In order to determine the differences in the downstream signals caused by these isoforms, we have developed cell lines stably expressing either RET9 or RET51 to compare the expression patterns of downstream target genes. Relative difference analysis was performed on RNA isolated from these cell lines, where cDNA from the RET9 expressing cells was limiting (tester) and cDNA from the RET51 cells was in excess (driver), to identify genes overexpressed in response to RET9. In preliminary analyses, we identified 6 differentially represented transcripts. Two of these genes have been independently confirmed by northern analyses (*ADAMTS1* and *HSGLUCOII*). The remaining 4 genes (*ILF2*, *GNB2*, *TAXREB107* and *DDB1*) are currently being investigated. Relevance of these genes to the different roles of these RET isoforms is being investigated.



**Detection of Genes Regulated by the Proto-oncogene *RET* Using Microarray Analysis.** *S.M. Myers*<sup>1</sup>, *G.C. Hui*<sup>1</sup>, *H.E. Feilotter*<sup>1,2</sup>, *L.M. Mulligan*<sup>1</sup>. 1) Dept Pathology, Queen's Univ, Kingston, ON, Canada; 2) Queen's University Microarray Centre, Kingston, ON, Canada.

The proto-oncogene *RET* codes for a receptor tyrosine kinase (RET) that plays an integral part in the development of the kidney and enteric nervous system. Splice variants of the *RET* transcript result in proteins with C-termini varying in the number of distinct amino acids; 9, 43 or 51 (RET9, RET43, RET51). These isoforms of RET are differentially expressed in the developing kidney and have been shown to be functionally different in *in vitro* assays. In order to determine if different genes are up or down regulated in response to the presence of these isoforms, we developed *RET* expression constructs for each of the RET isoforms and used these to generate cell lines stably expressing RET9 or RET51. Multiple independent RNA preparations from these cell lines were used to generate cDNAs labeled with either Cy3 or Cy5. Comparisons of gene expression in cells expressing RET9 versus RET51 or to RET9 versus untransfected cells were performed using microarrays containing both 1700 and 19,000 human cDNA clones. We selected genes with a two-fold difference in expression level for further study. Using this criterion, we have identified 14 genes differentially expressed between RET9 and RET51 expressing cells and 110 genes differentially expressed between RET9 and untransfected cells, including *RET* itself. We are presently verifying these results using more precise methods for comparison of gene expression. Our data suggest that a broad array of genes may be differentially expressed in response to RET activation. Relevance of up or downregulation of specific genes by RET is being investigated.

**Conditionally transformed human growth plate chondrocytes: A model system for the *in vitro* study of human chondrocyte biology.** *E.B. Mougey, J. Wang, R.C. Olney.* Dept Research, Nemours Children's Clinic, Jacksonville, FL.

The difficulty in obtaining and expanding human long bone growth plate chondrocytes to sufficient number to allow meaningful experiments, has hampered *in vitro* analysis of the mechanisms that regulate human chondrocyte proliferation and differentiation. While much has been learned about chondrocyte biology from numerous animal model systems, the more subtle aspects of human growth plate chondrocyte regulatory mechanisms await elucidation. One possible solution to the paucity of human cells would be a conditionally transformed cell line, which ideally would allow expansion and then reversion to the untransformed state. Indeed numerous primary human cell types have been successfully conditionally transformed; human costochondral chondrocyte and human osteoblast cell lines being the two most recent and relevant to this study. Therefore we sought to develop a conditionally transformed human long bone chondrocyte cell line. Human long bone growth plate cartilage was recovered from patients undergoing epiphysodesis after receiving informed consent. Chondrocytes were isolated by collagenase and hyaluronidase digestion and percoll gradient fractionation. The recovered chondrocytes were pooled, expanded and infected with adenovirus-*ori* SV40 *tsA* 209. This virus contains a temperature sensitive large T antigen mutant. Clones were obtained and expanded at 34°C and were verified as belonging to the chondrocyte lineage by assaying for collagen I, II, III, X, XI, and XII mRNA by RT-PCR. One clone (hGPC IV1B) exhibiting a doubling time of ~48 hr at permissive temperature and no growth at 40°C was selected for further study. The expression patterns of chondrocyte hypertrophy markers in monolayer and pellet cultures of this cell line suggest that it will be useful in studies of chondrocyte differentiation.

**Identification of human SRY-interacting factors.** *L. Salas-Cortes*<sup>1</sup>, *N. Hanley*<sup>2</sup>, *M. Fellous*<sup>1</sup>, *K. McElreavey*<sup>1</sup>. 1) Immunogenetique Humaine, Institut Pasteur, Paris; 2) 2Division of Human Genetics, University of Southampton, Southampton General Hospital, Southampton, U.K.

A number of human genes involved in sex determination have been identified in recent years including, SOX9, DAX1, FGF9 etc. However the vast majority of human cases of sex reversal (46,XY females with gonadal dysgenesis and 46,XX males) do not harbour mutations in these genes. Mutations involving the SRY gene occur in 15 % of cases of 46,XY females with complete gonadal dysgenesis and in about 1% of cases of 46,XY females with partial gonadal dysgenesis. This suggests that other, as yet unidentified genes, are involved in human sex determination. To identify coregulatory molecules that participate in transcriptional control by SRY, we applied a far-Western expression cloning strategy to identify transcripts encoding proteins that bind SRY. Initially a monoclonal antibody was raised against human SRY and immunohistochemistry demonstrated the presence of SRY protein in both Sertoli cells and germ cells. Extracts were prepared from human testis and, in a series of far-Western experiments using the human SRY protein as a probe and the antibody to detect interactions, we identified 6 nuclear proteins that specifically bind SRY. A lambda<sub>gt</sub>11 expression library from human adult testis was screened by far-Western blotting using recombinant SRY as a probe. 6 proteins were identified that bind SRY. A direct interaction between one of these factors and SRY was confirmed by co-immunoprecipitation experiments. This factor was demonstrated to be expressed in the human genital ridge at the moment of sex determination (48 days post-conception) by immunohistochemistry in pre-Sertoli cells and furthermore was expressed in the nuclei of Sertoli cells during fetal development and in adult testis.

**Position-specific expression of HOX genes along adult gastrointestinal tract in humans.** *N. Yahagi<sup>1</sup>, T. Suzuki<sup>1</sup>, T. Mitsuhashi<sup>1</sup>, R. Kosaki<sup>2</sup>, T. Takahashi<sup>1</sup>, K. Kosaki<sup>1</sup>.* 1) Dept of Pediatrics, Keio Univ, Tokyo, Japan; 2) Dept of Med Genetics, Saitama Children's Med Ctr, Iwatsuki, Japan.

Key molecules that control region-specific cellular renewal of the adult gut epithelium are yet to be delineated. We hypothesized that the 39 HOX genes, highly conserved transcriptional factors clustered on 4 separate chromosome loci, may represent plausible candidates because they are expressed in a segmental fashion along the gastrointestinal tract during embryonic period. HOX genes in each cluster are arranged in tandem and genes that are progressively more 5' within the clusters show progressively more anterior domains of expression in a Russian doll pattern. The purpose of the present study was to systematically investigate the expression pattern of the human HOX genes in tissues along the adult gastrointestinal tract: esophagus-stomach-duodenum-jejunum-ileum-ileocecum-cecum-ascending colon-transverse colon-descending colon-distally to rectum. A panel of cDNA derived from each tissue was amplified using primer pairs specific for each HOX gene. Monitoring of fluorescence from intercalating dye during the log-linear phase of the PCR reaction allowed quantification of relative expression level. We demonstrated the following: 1) HOXC genes were expressed at very low level. 2) Peak expression level of HOXA13, HOXA11, HOXA9, and HOXA6 were at descending colon, ascending colon, cecum, and jejunum, respectively. 3) Peak expression level of HOXB13, HOXB9, and HOXB7 were at rectum, descending colon, cecum, and jejunum, respectively. 4) Peak expression level of HOXD13 and HOXD10 were at rectum and cecum, respectively. Hence there is a correspondence between the physical location of the genes within the cluster and the relative expression along the anterior-posterior axis of the adult gut. This spatially co-linear expression pattern of the HOX genes was reminiscent of that during embryogenesis. We infer HOX genes may well play critical roles in position-specific differentiation of the adult gut epithelial precursor cells. Overlap of the expression domain indicates that HOXA, HOXB, and HOXD paralogues may have redundant functions in the regional specification of the gut.

***Prop1* and *Lhx4* interact in stimulating cell specification and expansion of the pituitary primordia.** *L.T. Raetzman, S.A. Camper.* Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI. 48109-0638.

Mutations in *PROPI*, *POUIF1* (*PIT1*) and *LHX3* (OMIM 601538, 173110, 600577) cause combined pituitary hormone deficiency (CPHD) in humans and mice. Most cases are associated with severe anterior pituitary hypoplasia due to differentiation failure of many cell types. Mice deficient in LIM homeobox transcription factor *Lhx4*, also have a hypoplastic anterior pituitary lobe, although no mutations in humans have been described to date (OMIM 602146). In the absence of *Lhx4* the somatotropes, thyrotropes and gonadotropes undergo initial specification, but fail in lineage specific proliferation. Little is known about the mechanism through which these homeobox genes promote the cytodifferentiation of specialized anterior pituitary cells. *LHX4* is expressed in many tissues during murine development, suggesting that it interacts with lineage specific factors to promote proliferation specifically in the pituitary. One likely candidate for this interaction is *PROP1*, a homeodomain transcription factor that has only been detected in the pituitary gland. To determine if *Lhx4* and *Prop1* have a complimentary role in lineage proliferation, we generated *Lhx4*<sup>-/-</sup>, *Prop1*<sup>df/df</sup> mice and analyzed the appearance of differentiated cells in the anterior pituitary. In the absence of both of these genes, the pituitary phenotype is more severe and the development is arrested earlier than in the single mutants. Rathke's pouch forms, yet there is no evidence of cell differentiation. Analysis of pituitary marker gene expression confirms the increase in severity of the double vs. single mutants. The  $\alpha$  subunit (CGA) and ACTH appear at e10.5-e12.5 and are the first hormone markers detectable in normal pituitaries and in the single mutants. In the double mutants the appearance of corticotropes is delayed until e16.5, at which point only a few differentiated cells are present. No other hormone producing cells are detected. These data indicate that both *Prop1* and *Lhx4* together are necessary for expansion of all anterior pituitary cell types. This suggests that interactions between *PROP1* and *LHX4* could contribute to endocrine abnormalities in humans.

**Mutational and functional analysis of *HESX1* within Septo-Optic Dysplasia (SOD).** D. McNay, K. Woods, J. Turton, D. Dattani. Institute of Child Health, London, UK.

Septo-Optic Dysplasia(SOD) is a variable developmental abnormality of the midline structures of the brain, classically resulting in hypoplasia of the optic nerves, the septum pellucidum and the corpus callosum in addition to dysgenesis of the pituitary gland. The homeobox gene *Hesx1* is a transcriptional repressor expressed at gastrulation within the anterior midline visceral endoderm, with subsequent expression in the prosencephalon and Rathke's pouch. *Hesx1* null mutant mice manifest a phenotype similar to SOD in man. This led to the successful search for causative *HESX1* mutations in patients with SOD. Here we report the findings of a follow up study of 574 patients with SOD. The cohort was screened for sequence variations within the coding region of *HESX1* using SSCP and HPLC heteroduplex analysis. A heterozygous S170L mutation was identified in a sib pair, both of whom demonstrate growth hormone deficiency(GHD) but only one of whom shows optic nerve hypoplasia, and also in an unrelated child with GHD and an ectopic posterior pituitary(EPP). The mutation occurs within a highly conserved motif and shows a dominant mode of inheritance with incomplete penetrance. *In-vitro* analysis demonstrates a 7-fold reduction in DNA binding, while *in-vivo* studies revealed no effect on repression. A heterozygous E149K mutation was identified in a child with GHD, EPP and anterior pituitary hypoplasia. The E149K variant lies at position 42 of the homeodomain, at the first residue of the third helix. This residue is highly conserved and we are currently investigating the function of this mutation. A N125S substitution has been identified in 5 Afro-Caribbean individuals with SOD. This substitution, at position 18 of the homeodomain, occurs at Hardy-Weinburg equilibrium within the Afro-Caribbean population, but has not been documented in Caucasian controls. N125S is associated with an altered conformation in *in-vitro* EMSA studies, with an increased affinity for the P3 consensus site, whilst repression is unaffected *in-vivo*. To conclude, coding region mutations of *HESX1* in association with SOD are rare but lead to valuable insights into the role of *HESX1* in normal development.

**Loss-of-function mutation in the CFC domain of TDGF-1 is associated with human forebrain defects.** *J.M. dela Cruz*<sup>1</sup>, *E. Roessler*<sup>1</sup>, *R.N. Bamford*<sup>1</sup>, *R.D. Burdine*<sup>2</sup>, *D. Donnai*<sup>3</sup>, *A.F. Schier*<sup>2</sup>, *M. Muenke*<sup>1</sup>. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Skirball Institute of Biomolecular Medicine, NYU, New York, NY; 3) Regional Genetic Service, St. Mary's Hospital (SM2), Manchester, UK.

Nodal signalling has been implicated in vertebral development of the midline, forebrain, and left-right axis asymmetry. At the onset of gastrulation, the TGF- $\beta$  ligand Nodal is expressed in dorsal mesoderm progenitors and, subsequently, in the left lateral plate mesoderm. Based on mouse models, mice null for Nodal lack a primitive streak, most mesoderm and die early in development. Transheterozygotes for Nodal and Smad2 survive gestation but succumb early to severe anterior midline and laterality defects. Importantly, Nodal signalling depends on the extracellular EGF-CFC family of proteins that by an unknown mechanism acts as an obligate cofactor for Nodal.

Members of the EGF-CFC family are characterized by an N-terminal signal sequence, an EGF-like motif, and a novel cysteine-rich region known as the CFC domain. Based on animal models, mutations in genes within this family create developmental anomalies that include defects affecting the orientation of the anterior-posterior axis, germ layer formation, and laterality. As a member of the EGF-CFC family, *Tdgf-1/Cripto* is implicated in embryonic development as well as in carcinogenesis. Previous mouse studies demonstrated that *Cripto* homozygous mutants generate a mispositioned anterior-posterior axis and lack also a primitive streak, node, and embryonic mesoderm.

Human *TDGF-1* has been evaluated for its potential role in forebrain development by SSCP and denaturing HPLC. Two unique missense mutations in *TDGF-1* have been identified in patients with sporadic holoprosencephaly that were not found in over 200 normal control chromosomes. Here we identify a mutation in the conserved CFC domain of TDGF-1 in a patient with midline anomalies of the forebrain. The mutant protein is inactive in a zebrafish rescue assay, indicating a role for *TDGF-1* in human midline and forebrain development.

**Aquaporin gene expression in craniofacial tissues.** *W. Wang<sup>1</sup>, X. Lu<sup>1</sup>, P.S. Hart<sup>2</sup>, N.P. Piesco<sup>1</sup>, M.C. Gorry<sup>1</sup>, T.C. Hart<sup>1,2</sup>.* 1) Oral Medicine, University of Pittsburgh, Pittsburgh, PA; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, PA.

The aquaporins (AQP) family of membrane channel proteins serve as selective pores through which water crosses the plasma membranes of many tissue and cell types. To date, 10 members of this transporter family have been cloned, designated AQP0-9. AQPs have different structures, which give them specific or selective function to be permeated by water, glycerol, and other small solutes. There are 2 subgroups of AQPs: water-selective channels (orthodox aquaporins) and channels permeated by water, glycerol, and other small molecules (aquaglyceroporins). The AQPs have distinct tissue distributions and different functions during water metabolism. We screened for all 10 AQPs in human gingiva (HG), fetal meckel cartilage (FMC) and fetal mandibular bone (FMB) by RT-PCR. Primers were designed to amplify specific AQPs and distinguish genomic and cDNA. RESULTS: AQP3 and AQP7 were expressed in HG; AQP1, AQP3, AQP4, AQP5 and AQP6 were expressed in FMC. Surprisingly, no AQPs were expressed in the FMB. AQPs have different permeabilities. AQP3 and AQP7 are aquaglyceroporins permeable to both glycerol and water. AQP3 is also permeable to urea. AQP1, AQP4, AQP5 and AQP6 are orthodox aquaporins. The expression of many AQPs in cartilage may reflect a high metabolic rate. As AQPs are expressed in a developmentally regulated fashion, it may be that at the particular stage we examined, no AQPs were expressed but at a different stage or after birth, they are expressed. While it is possible AQPs play no role in water transport in FMB, novel AQPs may be involved or other tissues may support water transport in FMB. Meckel cartilage is important for mandibular bone development and mineralization is part of this process. Lipid synthesis is reported to play a very important role during bone mineralization. AQP3 was expressed in Meckel cartilage. Thus, it may be involved in lipid metabolism and be important for bone mineralization. Further studies are needed to determine the developmental expression of the AQPs in both gingival and Meckel cartilage and also to determine their location in these tissues.



**Localization of *FOXC1* transcripts in normal adult human eye and optic nerve by *in situ* hybridization. R.**

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Mutations in the forkhead/winged helix family member *FOXC1* cause a spectrum of anterior segment eye defects that are related to the development of congenital glaucoma associated with chromosome 6p25, most notably Axenfeld-Rieger Anomaly. We have recently used an optimized donor eye preservation method and tissue RNA isolation procedure and demonstrated *FOXC1* expression in the trabecular meshwork and optic nerve head as well as other normal adult eye tissues by RT-PCR analysis. In this study, we used *in situ* hybridization to further refine the analysis of *FOXC1* expression in specific ocular tissues associated with glaucoma pathogenesis. In the anterior segment of the normal adult human eye, *FOXC1* expression was noted in the trabecular meshwork, ciliary muscle, and corneal epithelium. In the optic nerve head and optic nerve, *FOXC1* transcripts were abundantly expressed in the dura mater and arachnoid layers of the meningeal sheath; and, to a lesser extent, in optic nerve axons. *FOXC1* expression in the trabecular meshwork, ciliary body and optic nerve suggest that *FOXC1*, a gene known to be associated with developmental glaucoma, may have an important role in the adult eye.

**Specific decrease in fibrillin-1 and fibrillin-2 expression following antisense targeting in chick embryos. M.**

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Mutations in fibrillin-1 and fibrillin-2, microfibrillar glycoproteins with unique and overlapping distributions, are known to cause the Marfan syndrome (MFS) and congenital contractural arachnodactyly (CCA), respectively. Given the pathophysiology of these disorders, it has been hypothesized that fibrillin-1 plays primarily a "load bearing" function in the extracellular matrix while the function of fibrillin-2 may be more as a director of elastogenesis. Importantly, both fibrillins are expressed in the early developing embryo where "load bearing" is probably not critical.

We have successfully used phosphorothioate antisense oligonucleotides bound to cationic block copolymers of polyethyleneglycol and polyethylenimine to enter efficiently into early chick embryos. Embryos have been successfully inoculated in vitro and in ovo from 24 to 120 hours of fertilization. Sense controls were included in all experiments.

Inoculation with both antisense to fibrillin-1 and fibrillin-2 causes a delay in embryonic development. While this delay is variable, in all cases, there is a significant decrease in the size of the vascular net. In addition, the morphology of the blood vessels in the vascular net is abnormal. This may contribute to the delay in development of the embryos.

To examine the specificity of the targeting we used immunostaining with antibodies to chick fibrillin-1 (gift of Dr. R. Burke) and fibrillin-2 (gift of Dr. C. Little). When antisense to fibrillin-1 was inoculated into the embryos we observed a decrease in expression of fibrillin-1, but normal fibrillin-2 immunostaining. The converse was observed following inoculation of antisense to fibrillin-2. These studies highlight our ability to deliver antisense oligonucleotides with good specificity. In addition, they suggest a possible antiangiogenic affect on the vascular net of the chick embryo.

**In search of placenta-specific genes and promoter elements.** *C. Galaviz-Hernandez, T. Tanaka, M. Ko, M. Uda, D. Schlessinger, R. Nagaraja.* Laboratory of Genetics, National Institute on Aging, Baltimore, MD.

Responsible for gas and nutrient exchange between mother and fetus, the placenta is thus indispensable for proper development. Some genes have been detected that are critical for placental function, based on gene disruption studies in mice and differential gene expression studies in choriocarcinoma cells. We have extended the search for placental-specific genes in two ways. In one, based on genomic sequence, we found PLAC1, distal to HPRT in Xq26, and specifically expressed in placenta in 7.5 to 14.5 dpc in ectoplacental cone, giant cells, and labyrinthine trophoblasts. To try to understand the specificity of expression in the placenta, we have cloned fragments of genomic DNA upstream of PLAC1. Constructs have been made that place putative promoter fragments 5' of a reporter luciferase gene, and they are being tested in the JEG-3 and BeWo cell lines (which show endogenous expression of placental genes) along with HeLa cells as a negative control. A TATA box found at 30 bp and a PEBP2/CREBdelta binding site at 647 nucleotides are the only transcription elements found thus far, but they, in the context of 3.6 kb of DNA upstream of exon 1 of the gene, are insufficient to give specific expression. Longer fragments are now being tested. In a second approach, we have used RNA isolated from 12 dpc placenta as a probe on microarrays containing the NIA mouse 15K cDNA set. For a group of 6 cDNAs, an expression pattern suggesting placental specificity was confirmed by Northern blot and in situ hybridization experiments, and two of the cDNAs have been characterized in detail. Comparative sequence analysis of possible promoter elements shared with PLAC1, however, awaits the availability of the corresponding genomic regions.

**Vax2 inactivation in mouse determines alteration of the eye dorsal-ventral axis, misrouting of the optic fibers and eye coloboma.** *S. Banfi<sup>1</sup>, A.M. Barbieri<sup>1</sup>, V. Broccoli<sup>1</sup>, P. Bovolenta<sup>2</sup>, G. Alfano<sup>1</sup>, A. Marchitello<sup>1</sup>, A. Ballabio<sup>1</sup>.* 1) Telethon Institute of Genetics and Medicine (TIGEM), Naples, Italy; 2) Departamento de Neurobiología del Desarrollo, Instituto Cajal, CSIC, Madrid, Spain.

Vax2 is a homeobox gene whose expression is confined to the ventral portion of the prospective neural retina. Overexpression of this gene at early stages of development in *Xenopus* and in chicken embryos determines a ventralization of the retina, thus suggesting its role in the molecular pathway underlying eye development. Here, we describe the generation and characterization of a mouse with a targeted null mutation of the Vax2 gene. Vax2 homozygous mutant mice display incomplete closure of the optic fissure that leads to eye coloboma. This phenotype is not fully penetrant suggesting that additional, non-genetic factors contribute to its generation. Vax2 inactivation determines dorsalization of the expression of mid-late (EphB2 and ephrin-B2) but not early (Pax2 and Tbx5) markers of dorsal-ventral polarity in the developing retina. Finally, Vax2 mutant mice exhibit abnormal projections of ventral retinal ganglion cells. In particular, we observed the almost complete absence of ipsilaterally projecting retinal ganglion cells axons in the optic chiasm and alteration of the retinocollicular projections. All these findings indicate that Vax2 is required for the proper closure of the optic fissure, for the establishment of a physiological asymmetry on the dorsal-ventral axis of the eye and for the formation of appropriate retinocollicular connections.

**Dissection of the regulatory elements that control the *Chx10* pathway of eye development.** R. Abboud<sup>1</sup>, A. Sertié<sup>1</sup>, S. Basu<sup>2</sup>, B.E. Snow<sup>1</sup>, D.J. Horsford<sup>1</sup>, M. Burmeister<sup>2</sup>, R.R. McInnes<sup>1</sup>. 1) Program in Developmental Biology, Research Institute, Hospital for Sick Children, Toronto, ON, Canada; 2) Mental Health Research Inst., Depts. of Psychiatry and Human Genetics, Univ. of Michigan, Ann Arbor, MI.

The *Chx10* gene encodes a homeodomain transcription factor essential for eye development. In mouse, *Chx10* expression is first detected in retinal progenitor cells, but is later restricted to the inner nuclear layer of the adult retina. *Chx10* is also expressed in the hindbrain, suggesting that it may be regulated by several enhancers. To identify the regulatory elements that control *Chx10* retinal expression, we are using two complementary strategies. First, we established, by PFGE analysis, that one *Chx10* mutant allele, *or<sup>2J</sup>*, is due to a 75-100 kb rearrangement located 10-20 kb upstream of the *Chx10* transcription unit; this unit is intact in *or<sup>2J</sup>*, as shown by Southern blots, SSCP analysis and DNA sequencing. By *in situ* hybridization, we found that the *or<sup>2J</sup>* allele ablates the expression of *Chx10* in retinal progenitors from E9.5-E12.5, whereas hindbrain expression is unaffected. Using transgenic mice, we also found that 5.3 kb of sequence immediately 5' of the *Chx10* transcription unit allows normal *Chx10* expression in hindbrain but not in retina, at E10.5. Second, to identify putative regulatory elements in the DNA sequences ~100 kb upstream of *Chx10/CHX10*, we aligned these sequences from mouse and human and identified 31 shared elements larger than 100 bp and with more than 80% identity. Initial studies show that one element (#510) identifies abnormal bands in blots of *or<sup>2J</sup>* DNA, and cannot be amplified from *or<sup>2J</sup>* DNA using some primer pairs. We conclude that i) the *or<sup>2J</sup>* allele appears to contain a rearrangement that disrupts the element(s) controlling *Chx10* developmental retinal expression; ii) the retinal regulatory element(s) may be found in the set of 31 conserved sequences upstream of the gene; iii) element #510 may be disrupted in the *or<sup>2J</sup>* allele; iv) the regulatory elements that direct *Chx10* hindbrain expression are distinct from those for the retina and are contained in 5.3 kb 5' to the transcription unit.

**Developmental patterns of altered gene expression in the lungs of a glucocorticoid receptor knockout mouse:**

**Altered expression of the retinoic acid responsive growth factor midkine.** *F. Kaplan<sup>1</sup>, J. Comber<sup>1</sup>, T. MacRae<sup>1</sup>, R. Sladek<sup>2</sup>, T. Hudson<sup>2</sup>, N.B. Swezey<sup>3</sup>.* 1) Dept Human Genetics/Pediatrics, McGill U / McGill U Health Centre Montreal, QC; 2) Montreal Genome Centre; 3) Hospital for Sick Children Toronto, ON.

Deficiency of lung maturation due to premature birth is a leading cause of neonatal morbidity (respiratory distress syndrome, RDS.) Normal maturation of the lung is dependent on glucocorticoid (GC) stimulation. We are investigating intermediary mechanisms that link GC stimulation to the timely achievement of a mature lung in a GC receptor (GR) knockout mouse. Seventy-five percent of mice homozygous for a hypomorphic disruption in the GR gene ( $GR^{hypo}$ ) die shortly after birth of respiratory failure. Surviving  $GR^{hypo}$  mice are normal and fertile. We used cDNA microarrays and northern analysis to identify genes that are differentially expressed in the lung at fetal day 18 ( $GR^{hypo-18}$ ) and postnatal day 1 in  $GR^{WT}$  vs  $GR^{hypo}$  mice. Neonatal animals were divided into survivors ( $GR^{hypo/surv}$ ) and non-survivors ( $GR^{hypo/nsurv}$ ). Multiple alterations in gene expression were observed in the 3 groups of  $GR^{hypo}$  vs  $GR^{WT}$  mice. The retinoic acid responsive gene Midkine (MK), was among 8 genes identified with similar patterns of altered gene expression in all 3 groups. MK is upregulated in the lungs of  $GR^{hypo-18}$ ,  $GR^{hypo/nsurv}$  and  $GR^{hypo/surv}$  mice. By contrast, MK is downregulated in  $GR^{hypo}$  mice at fetal day 16.5. In rat lung cell culture, MK expression is enriched in epithelium compared to mesenchyme from fetal day 16.5-21. GC treatment downregulated rat lung epithelial MK expression at day 21. MK, a secreted heparin binding growth factor, is expressed at high levels in mid-gestation and is associated with epithelial-mesenchymal interactions in the developing lung. MK is important in angiogenesis and tissue remodeling. MK is not detected in adult rat lung but is expressed in lung tumours. Our findings suggest that absence of a functional GR is associated with inhibition of the downregulation of MK by GC in late gestation. Overexpression of MK in late gestation, in turn, is associated with deficient terminal differentiation of the fetal lung.

**LGL1, a developmentally and glucocorticoid regulated gene, modulates airway branching morphogenesis. L.**

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We recently reported (*Am J Physiol.* 1999 Jun; 276(6 Pt 1): L1027-36) a novel gene, late gestation lung 1 (LGL1). LGL1, a member of the CRISP family of cysteine-rich secreted proteins, is glucocorticoid inducible, developmentally regulated, and expressed in the mesenchyme, but not epithelium, of fetal rodent and human lung. Here we report mapping of LGL1 to human chromosome 16q24 by FISH. We investigated LGL1 subcellular localization and function in fetal lung development. LGL1 mRNA is detectable from gestational day 12 through to maximal expression on gestational day 21 (term = d 22). Lgl1 protein expression is maximal in late gestation (days 18 - 21). Consistent with the postulate that lgl1 protein is secreted, lgl1 fused with green fluorescent protein was localized by confocal microscopy to the cytoplasm, but not the nuclei, of fetal rat lung adjacent fibroblasts. Expression of LGL1 mRNA (by in situ hybridization) and lgl1 protein (by immunohistochemistry) is evident in mesenchymal cells during the pseudoglandular stage of lung development, when branching morphogenesis begins. To test the hypothesis that lgl1 modulates airway branching morphogenesis, fetal rat day 13 lung explants were treated for 24 and 48 hr with LGL1 antisense oligodeoxynucleotides. The number of terminal airway buds ( $p < 0.001$ ), and the levels of LGL1 mRNA and lgl1 protein ( $p < 0.01$ ), were significantly reduced in the antisense treated explants when compared to sense, missense and untreated controls in a dose and time dependent fashion. Our findings implicate lgl1 in mesenchymal-epithelial interactions that regulate airway branching. Furthermore, the timing of maximal lgl1 protein expression during active alveolarization leads us to speculate that lgl1 may also promote alveolar formation. This work was supported by the Canadian Institutes of Health Research.

**DIFFERENTIAL GENE EXPRESSION IN NASCENT AND MATURE MOUSE OVARY ASSESSED BY 15K cDNA MICROARRAY.** *L.M. Herrera, M.S.H. Ko, D. Schlessinger.* Laboratory of Genetics, National Institute on Aging, National Institutes of Health, Baltimore, MD.

Reproductive lifespan is set by the development of the ovary, with all of the eggs and follicles that a woman will have in adult life already formed at the time of her birth. The progressive utilization of follicles, particularly after puberty, leads to menopause at an average age of 51; but in about 1% of women the rate of attrition of oocytes and follicles in utero is too great, leading to premature ovarian failure. To analyze the basis for the number of follicles, one approach aims to discover the genes and regulatory networks involved in ovarian and follicle development. The mouse provides a particularly good model system, because the phases of oocyte, follicle, and ovary development are discretely phased. We have used cohorts of cDNAs recovered from every mouse embryonic cleavage stages, selected fetal tissues and organs, and newborn ovary. In particular, 7,000 ESTs from newborn ovary have been sequenced and analyzed for the frequency of representation of individual genes. Single members of each gene observed (3,892) have been included in an initial group of 15,000 distinct genes in the NIA15K cDNA microarray. Using the NIA15K cDNA microarray we have compared gene expression in newborn (nascent) and adult (mature) ovary, and have extended the comparison to 12.5 dpc placenta, 12.5 dpc embryo, and adult testis, to find ovary-specific genes and germ cell-specific genes. Results were verified for sampled genes by Northern analysis and real-time PCR, and several genes were shown to have specific follicular or germ cell localization, as confirmed by in situ hybridization. Both nascent and mature ovary show very high expression of synthetic metabolic genes, including those encoding energy-yielding and protein synthetic machinery. In addition, there were also differentially expressed genes, notably including several transcription factors.



**Expression of Three Proneural basic Helix-Loop-Helix Transcription Factors in Extravillous Trophoblasts suggests Role in Trophoblast Differentiation and Invasion.** *C.B.M. Oudejans, B.A. Westerman, A. Poutsma, I.J. van Wijk.* Clinical Chem, 'Vrije Universiteit' Medical Center, Amsterdam, the Netherlands.

Trophoblasts form the outermost region of the developing embryo and contact the uterus during implantation. The contact between the maternal cells of the uterus and trophoblast cells is further established when differentiated trophoblasts invade interstitially into the uterus, a process which occurs in humans, but not in rodents. In human placentation, villi are formed from week 4 to 20. On the top of the anchoring villi, proliferative stem cells downregulate proliferation, leave the villous tip and adopt an extravillous phenotype. These extravillous trophoblast cells (ETCs) invade into the maternal decidua and myometrium. This invasion has similarities to invasion by cancer cells. However, trophoblast invasion is restricted in time and place, in contrast to tumor invasion. Since differentiation of trophoblasts is controlled genetically by basic Helix-Loop-Helix (bHLH) transcription factors, we are using invasive placenta cells to study bHLH regulated differentiation, and use this as a model system to investigate the process of tumor invasion. We constructed a unique cDNA library of placental bed tissue obtained during week 10 of gestation enriched for ETCs in different stages of invasion. A subset of proneural genes (i.e. NEUROD1, NEUROD2, ATH2) was found to be activated in extravillous trophoblast cells, as is the Achaete Scute member ASCL2 (HASH2). Other (pro)neural genes of the Achaete Scute and Atonal family (all genes currently known were tested) were found not to be expressed in placental bed tissue. Thus, out of 9 proneural genes tested, 3 genes, all belonging to the same subfamily, are transcribed in placental bed tissue. This suggests the use of a differentiation cascade by trophoblast analogous to the cascade used by neuroectoderm involving Mash1. So far, the only other tissue besides neuroectoderm in which transcription of proneural bHLH genes has been found to be essential is the pancreas. Current experiments are being performed to see whether subpopulations of cells can be identified with differential (co)expression of these 3 bHLH genes in invasive cells.

**Refinement of Lif-Stat-3 Pathway Using Microarray Analysis.** *S.A. Jaradat<sup>1</sup>, T. Yokota<sup>2</sup>, M.S.H Ko<sup>1</sup>*. 1) Lab Genetics, NIH/NIA, Baltimore, MD; 2) Institute of Medical Science, The University of Tokyo, Minatoku, Tokyo 108-8639, Japan.

Mouse embryonic stem (ES) cells are pluripotent and can differentiate into many different cell types in vitro. The ES cells can be propagated in an undifferentiated state in vitro in the presence of leukemia inhibitory factor (LIF). Although it is well established that LIF exerts its function by activating a Stat3 transcription factor, its downstream targets are largely unknown. To identify such genes, we have employed cDNA microarray technology. First, the STAT3ER construct was made by fusing the entire coding region of Stat3 with the ligand-binding domain of the estrogen receptor. Then the construct was stably transfected into ES cells. STAT3ER is conditionally active in the presence of the synthetic ligand 4-hydroxytamoxifen (4HT), and maintains ES cells in an undifferentiated state. ES cells were cultured in the presence or the absence of 4HT for up to 6 days. Total RNAs were extracted from these cells and utilized for NIA 15K cDNA microarrays hybridization. Student's t-test was applied to hybridization results done in triplicate. 500 genes showed statistically significant differences in expression between ES cells (4HT+) and ES cells (4HT-). Functionally these genes can be organized into five categories. The first category contains genes involved in cell cycle progression and DNA repair, including MutS, Chek1 and Cyclin D1. Genes in the second category include Igf-Bp3 and Caspase-8, known to function in apoptosis. Genes in the third category include p300/CBP acetyltransferases and Sfm1b Polycomb, which are known to function as global activators or repressors of transcription. Genes in the fourth category include Mef2 and Cytokeratin type II, which are tissue or lineage specific factors. The last category includes over 100 newly identified genes. Further analysis of these groups of genes should define further the role of Stat3 in the pluripotency and self-renewal of ES cells.

**Identification and Characterization of DAZ Function at Two Critical Stages in Germ Cell Development.** *F.L. Moore, J. Jaruzelska, M. Castillo, R.A. Reijo-Pera.* OB/Gyn/RS, UC San Francisco, 513 Parnassus ave., bldg HSW, rm. 1475 San Francisco, CA. 94143.

The identification of the DAZ (Deleted in Azoospermia) gene, the most genetically defined cause of infertility in males, has enhanced the possibility of understanding human germ cell development at the molecular level. The expression pattern of DAZ is restricted to germ cells in the testis and the protein sequence contains a RRM motif, which belongs to a family of RNA binding proteins. We are further characterizing the function of the DAZ gene by understanding what proteins interact with it. Seven interacting protein candidates have been identified using the yeast two-hybrid approach. Co-immunoprecipitation in yeast has verified an *in vitro* protein interaction between DAZ and Pumilio proteins and DAZ and BOL proteins. The Pumilio protein has been shown in *Drosophila* to function as a translational repressor. The Pumilio protein contains eight Puf (Pumilio Fbf) repeats that are used to bind RNA, and binds to the 3' UTR region of various transcripts to repress translation. The Pumilio protein has multiple roles during development and interestingly is essential for the maintenance of germ line stem cells in *Drosophila* oogenesis. The BOL gene, which is a new member of the DAZ gene family has been characterized in *Drosophila* as a translational activator and functions as a meiotic regulator in male germ cells. The DAZ and Pumilio proteins localize to the nucleus in spermatogonia and relocate to the cytoplasm in spermatocytes. The BOL protein is expressed at meiosis and localizes with the DAZ protein to the cytoplasm of spermatocytes. We believe that the DAZ protein has two functions during germ cell development. It plays a role in the maintenance of germ cells and is also required as a meiotic regulator. Further characterization of the DAZ protein and interacting proteins will lead to a better understanding the function of the DAZ gene family in germ cell development.

**The Kallmann syndrome gene homologue in *C. elegans* is involved in epidermal morphogenesis and neurite branching.** *E.I. Rugarli<sup>1</sup>, E. Di Schiavi<sup>2</sup>, M. Hilliard<sup>2</sup>, S. Arbucci<sup>2</sup>, C. Ghezzi<sup>1</sup>, A. Facciolli<sup>2</sup>, A. Ballabio<sup>1</sup>, P. Bazzicalupo<sup>2</sup>.* 1) TIGEM, Naples, Italy; 2) IIGB, Naples, Italy.

Kallmann syndrome (KS) is an inherited disorder defined by the association of anosmia and hypogonadism and is due to impaired targeting and migration of olfactory axons and of gonadotropin-releasing hormone secreting neurons. The gene responsible for the X-linked form of Kallmann syndrome (KAL) has been identified and encodes a secreted protein of still elusive function. It has been proposed that KAL might be involved either in olfactory axon target recognition and/or invasion or in olfactory bulb morphogenesis. Functional characterization of KAL has been hampered so far by the failure to clone a mouse homologue. We now describe the isolation of the KAL homologue in *Caenorhabditis elegans*. By analyzing loss of function and hypermorph mutants of the *C. elegans* *kal-1* gene, we provide evidence that CeKAL-1 is involved in neurite extension/branching and in epidermal morphogenesis during ventral enclosure and male tail formation. Since *kal-1* reporters are expressed in neuronal cells while the cells most affected by mutants are epithelial cells undergoing active morphogenesis, we propose that CeKAL-1 acts non-cell-autonomously to modulate the adhesion of cells and growth cones to the matrix and to other cells, in concert with other molecules. Likely candidates are components of the Eph-Receptor/ephrin and/or semaphorin pathways. We also demonstrate that overexpression of worm and human KAL cDNAs in the nematode result in the same phenotypes, indicating functional conservation across species and establishing the nematode as a powerful model to investigate the mechanism of action of the KAL protein and to identify interacting partners. These results provide the first *in vivo* evidence of the function of KAL gene and open new perspectives on the pathogenesis of KS. Furthermore our data contribute to the emerging knowledge that the same set of molecules underlies both morphogenesis and axonal-neuronal navigation in vertebrates and invertebrates.

**Deficiency of *Zic3* causes defects in gastrulation and left-right patterning.** *S.M. Ware, S. Purandare, B. Casey, J.W. Belmont.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

X-linked heterotaxy (HTX1) is a rare developmental disorder characterized by disturbances in laterality and other midline developmental field defects. HTX1 results from mutations in *ZIC3*, a member of the *GLI* transcription factor superfamily, which is expressed in the primitive streak, dorsal central nervous system (CNS), tailbud and developing limb. A targeted deletion of the murine *Zic3* locus has been created in order to investigate its function and to determine how it interacts with other molecular components of the left-right axis pathway. The phenotype of these mice closely models the defects seen in human HTX1 with malformations in liveborn null mice including complex congenital heart disease, pulmonary reversal or isomerism, CNS defects and vertebral and rib anomalies. Embryonic lethality is seen in approximately 50% of null mice with an additional 30% lethality in the perinatal period. Analyses of embryos at 7.5-9.5 days post coitum show varying degrees of gastrulation defects. The most severely affected embryos have no obvious node by both gross morphologic examination and analyses with molecular markers. Less severely affected embryos show defective mesoderm development including notochord abnormalities and degeneration and failure of somitogenesis. Failure of neural tube closure is found in a subset of embryos and it is unclear whether this represents a primary failure or is secondary to abnormal mesodermal inductive signals. A third category of embryos include those in which gastrulation occurs but left-right axis development is disrupted. Examination of these null embryos shows defects in embryonic turning, cardiac morphogenesis and positioning, and pulmonary and abdominal situs. Investigation of *nodal* expression in *Zic3* deficient mice indicates that although *nodal* is initially expressed symmetrically at the node, there is failure to maintain expression and to shift to asymmetric expression. Taken together, these results suggest that *Zic3* is required for maintenance of the node and that its expression plays a critical role both in gastrulation and left-right patterning.

**Microarray transcriptional profiling of DNA repair-related genes in mammalian oocytes.** *A.J. Wyrobek<sup>1</sup>, M.A. Coleman<sup>1</sup>, S. Mabery<sup>1</sup>, B.J. Marsh<sup>1</sup>, R. Raja<sup>2</sup>, T.B. Taylor<sup>2</sup>, F. Marchetti<sup>1</sup>.* 1) Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; 2) Arcturus, Mountain View, CA.

Maintenance of genomic integrity at fertilization and during early development relies on maternally stored gene products until the time of genomic activation. Expression microarray technology provides a genomic approach to characterizing the initial storage pool and subsequent changes in transcript levels after fertilization. However, the low amount of mRNA in oocytes has remained a serious obstacle. Using the mouse model, we have improved the technology for obtaining RNA and preparing antisense RNA (aRNA) from oocytes for microarray evaluation. We utilized custom-built cDNA microarrays containing over 800 genes involved in DNA damage recognition, DNA repair, cell-cycle control, stress response and various related functions. Oocytes free of contaminating cumulus cells were collected from superovulated B6C3F1 females 16 hr after induction of ovulation. RNA was isolated and amplified by a new in vitro linear amplification process (RiboAmp<sup>TM</sup> RNA Amplification Kit, Arcturus). An initial pool of 84 oocytes containing ~35 ng of total RNA yielded ~11 mg of aRNA. Five mg aliquots of aRNA were labeled and hybridized to our array. Approximately 150 genes, including *Cyclin A*, *Cenp B*, *Cdc2*, *Nbs1* and *Bax*, showed significant intensities above the confidence intervals for negative reference genes. The microarray findings for selected DNA-repair-related genes were confirmed by RT-PCR and/or TaqMan (e.g., *Ku80*, *Rad51*, *Rad54*, *Dna-pkcs*, *Xpa*, *Ape1*). Nineteen transcripts that represented unannotated ESTs from developmental libraries were also detected on our microarrays: four of these encode putative proteins that have human and *C. elegans* homologies. These microarray protocols are being optimized to require lower amounts of RNA from fewer oocytes and to characterize the expression profiles during early development. [This work was conducted under the auspices of DOE by the University of California, LLNL under contract W-7405-ENG-48 with support from NIH ES09117-03, DOE KP110202 and TRDRP 7RT-0073.].

**Microarray transcriptional profiling of mammalian male meiosis.** *L.M. Tomascik-Cheeseman*<sup>1,2</sup>, *F. Marchetti*<sup>1</sup>, *M.A. Coleman*<sup>1</sup>, *E. Yin*<sup>1</sup>, *K. Rankin*<sup>1</sup>, *J. Nath*<sup>2</sup>, *A.J. Wyrobek*<sup>1</sup>. 1) Biology & Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA; 2) Genetics and Developmental Biology Program, West Virginia University, Morgantown, WV.

The first wave of spermatogenesis in the prepubertal mouse provides a unique model for investigating the kinetics of gene expression during mammalian spermatogenesis. The purpose of this research was to characterize the patterns of gene expression at the onset and during the progression of male meiosis. Testes were evaluated at specific postnatal days when the leading edge of the first wave of spermatogenesis was in the preleptotene (day 9) and pachytene (day 14) stages, respectively. Total RNA was isolated from seminiferous tubules, fluorescently labeled and hybridized onto oligonucleotide microarrays. Hundreds of genes were significantly and/or differentially expressed across meiosis. Several distinct expression patterns were observed: (a) genes known to be essential for normal spermatogenesis were differentially expressed between the preleptotene and pachytene stages [e.g., *Mdes* was down-regulated and *Dazl* was up-regulated]; (b) genes with no previously known role in spermatogenesis, meiosis or fertility were differentially expressed at the two stages [e.g., *Crkol* and *Peg3* were down-regulated; *Grp78* and *Tcf20* were up-regulated]; and (c) genes that did not have detectable levels of expression at the onset of meiosis were significantly expressed during the pachytene stage (e.g., *Tcte3*, *Pem* and *Rzf*). These findings provide important insights into the kinetics of gene expression during spermatogenesis, identify novel meiosis-specific and meiosis-enriched genes, and assign new putative functions to known genes. This research enhances our understanding of the mechanisms of meiosis and the genetic causes of male infertility. [Work was conducted under the auspices of U.S. DOE by the University of California, LLNL under contract W-7405-ENG-48 with support from NIH ES09117-02, NIEHS Superfund P4ZES04705, DOE KP110202 and West Virginia University.].

***NNAT* resides in a micro-imprinted domain on human chromosome 20q11.2.** *H.K. Evans*<sup>1,2</sup>, *A.A. Wylie*<sup>1</sup>, *S.K. Murphy*<sup>1</sup>, *R.L. Jirtle*<sup>1</sup>. 1) Department of Radiation Oncology, Duke University, Durham, NC; 2) University Program in Genetics, Duke University, Durham, NC.

A small proportion of the genome consists of imprinted genes - genes expressed exclusively from one parental allele. Imprinted genes are involved in the etiology of a variety of clinical diseases including Prader-Willi, Angelman, and Beckwith-Wiedemann syndromes. In addition, they have been implicated in several complex disorders such as diabetes and autism. Yet despite the clinical significance of imprinted genes, the precise regulatory mechanism for this phenomenon remains undefined. Therefore, our laboratory was interested in identifying novel imprinted genes in humans that could be used as models to further study and understand imprinting. This study focuses on characterizing the imprinting at one specific locus - *Peg5/NNAT*. While previous research has confirmed imprinting of mouse *Nnat*, the imprinting status of human *NNAT* has not been addressed. Using gene-specific polymorphisms to determine biallelic versus monoallelic expression of *NNAT* in human fetal tissues, we demonstrate that *NNAT* is imprinted in humans. Since differential methylation of CpG islands near or within imprinted genes has been demonstrated to play a role in the imprinting mechanism, we used bioinformatics in combination with bisulfite treatment and subsequent sequencing of genomic DNA to perform an in-depth methylation analysis of the *NNAT* locus. We identified a large CpG island at the *NNAT* promoter that exhibits consistent differential methylation in all tissues examined and hypothesize that this island may be a component of an imprint control region for the *NNAT* domain. Furthermore, we show that *NNAT* lies within the singular 8.5 kb intron of a second gene, *BLCAP*, which is not imprinted. Therefore, *NNAT* lies in an imprinted micro-domain, making it uniquely suited for investigating mechanisms of localized imprint regulation.



**Fibroblast Growth Factor Receptor 1 signalling during limb and sternum development.** *M.D. Lalioti<sup>1</sup>, A.O.M. Wilkie<sup>2</sup>, J.K. Heath<sup>1</sup>*. 1) Sch Biosciences, Univ Birmingham, Birmingham, UK; 2) Weatherall Institute of Molecular Medicine, University of Oxford, The John Radcliffe, UK.

The fibroblast growth factor receptors (FGFRs1-4) are a family of related transmembrane tyrosine kinases, which, upon binding of the fibroblast growth factors (FGFs), dimerize, autophosphorylate, and initiate intercellular signaling. A Pro252Arg on the ligand-binding domain of R1, and the same alteration in R2 cause Pfeiffer and Apert syndromes, respectively. Ser252Phe and Ser252Trp in R2 also cause Aperts, while Ser252Phe/Pro253Ser cause Pfeiffer syndrome. Both syndromes are characterized by various degrees of craniosynostosis and limb abnormalities. Taking into consideration the complex pattern of alternative splicing of FGFR1-3 and the autosomal dominant mode of inheritance of the syndromes, we created mouse models by introducing FgfR1 BAC transgenes harboring specific point mutations. A mouse containing a wt BAC was normal, showing that overexpression of FgfR1 is not responsible for the disease. Mice containing the Ser251Phe/Pro252Ser show limb, skull, sternum and tail deformities. Smaller body size, preaxial polydactyly and/or triphalangial big toe occur in 100 % of the animals. This is consistent with the big toe defect of the Pfeiffer patients due to Pro252Arg. Other defects include fusion of sternebrae and rib misalignment, shorter digits, kinked tails, and forelimb autopod abnormalities. The phenotype is copy number dependent. The defects likely to be due to a gain of function of the mutant receptor, presumably due to increased affinity for specific Fgf ligands, as it has been previously shown in vitro. Our models provide a powerful system for dissecting the role of Fgfr1 during development of the limbs, skull and sternum, their affinity for Fgfs and their signalling to downstream morphogens.

**Analysis of *Dmrt1* in *Ellobius lutescens*: Evidence for the exclusion of a testis inducing function.** A.C. Baumstark<sup>1</sup>, W. Vogel<sup>1</sup>, A. Schulze<sup>1</sup>, M. Akhverdyan<sup>3</sup>, I. Reiser<sup>2</sup>, W. Just<sup>1</sup>. 1) Dept Human Genetics, Univ of Ulm, Ulm, Germany; 2) Dept Anatomy and Cellular Biology, Univ of Ulm, Ulm, Germany; 3) Dept Conservation Biology and Genetics, Uppsala Univ, Uppsala, Sweden.

Mammalian sex determination is a complex event involving various genes. Animal models are a suitable tool to investigate these genes. In the mole vole *Ellobius lutescens* both sexes show the identical karyotype 17,X. Cytogenetic analyses of a polymorphism in different *E. l.* families revealed sex specific segregation of chromosome 1 indicating the localization of a sex determining gene. The absence of *SRY*, initiating testis differentiation in other mammals has been shown. Thus it is hypothesized that another gene from the sex determination cascade is mutated and has taken over testis determining function. *SOX9*, for which a testis determining function in the absence of *SRY* has been shown, has been excluded from being the testis initiating factor in *E. l.* Another candidate gene for the induction of testis development is *DMRT1*. *DMRT1* shows testis specific expression in all vertebrate species examined to date. Because of its localization on the Z chromosome in birds it is considered a candidate gene for the postulated dosage dependent avian sex determination. A comparable mechanism, based on two *DMRT1* alleles differing in expression levels, could also exist in *E. l.* To investigate the role of *Dmrt1* in testis determination of *E. l.* we isolated and sequenced phage clones containing part of the gene. One of these clones was used to localize *Dmrt1* on distal 1p outside the candidate sex determining region on 1q by FISH. Intronic sequences were screened for polymorphic markers by an enzymatic mutation detection system. Analysis of the distribution of alleles of isolated markers showed no association of alleles with the sex of the tested animals. Because of the expected extensive linkage disequilibrium in the investigated subpopulation and the close linkage between tested markers and *Dmrt1* an association between *Dmrt1* alleles and sex and thus a testis determining function of *Dmrt1* can also be excluded. This study was supported by grant Re 413/6-1 from the Deutsche Forschungsgemeinschaft.

**Identification of two *Drosophila* homologs of *Chx10*, a homeobox gene essential for eye development.** *T. Erclik*<sup>1</sup>, *G. Sheng*<sup>2</sup>, *K. Duecker*<sup>3</sup>, *C. Desplan*<sup>2</sup>, *E. Hafen*<sup>3</sup>, *H. Lipshitz*<sup>1</sup>, *R.R. McInnes*<sup>1</sup>. 1) Program in Developmental Biology, Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Biology, New York University, New York, N.Y., USA; 3) Zoologisches Institut, Universitat Zurich, Zurich, Switzerland.

*Chx10* encodes a homeodomain (HD) protein and is an essential component of a genetic pathway that regulates mammalian eye development. In humans and mice, homozygous *Chx10* null alleles are associated with impaired proliferation of retinal progenitors, absent bipolar cells, microphthalmia and blindness. Because regulators of eye development are highly conserved between invertebrates and mammals (e.g. *Pax6*), we are using the powerful genetics of *Drosophila* to facilitate the dissection of the mammalian *Chx10* regulatory pathway. We have identified two *Drosophila* homologs of *Chx10*, *dChx1* and *dChx3*. Both homologs are >75% identical to *Chx10* in the HD and CVC domain. *dChx1* and *dChx3* map to polytene region 5A3-6 on the X chromosome where they are located 33 kb apart. *dChx1* is expressed in the procephalic region and certain sensory neuron precursor cells in the embryo (like *Chx10*) while *dChx3* is not embryonically expressed. In third instar larvae, both *dChx1* and *dChx3* are expressed in the dorsal and ventral regions of the eye-antennal discs. *wingless*, a *Drosophila* *Wnt* homolog, is expressed in similar regions of eye-antennal discs where it functions to define the boundaries of the retinal field. Ectopic expression of *wingless* in the eye disc leads to ectopic expression of both *dChx1* and *dChx3* indicating that expression of the *dChx*'s may be activated in response to *wingless* signaling. Ectopic expression of *Chx10* or *dChx1* in the developing eye disc leads to a loss of photoreceptor cell types. This phenotype is consistent with the *dChx*'s functioning downstream of *wingless* to repress retinal cell fate at the boundary of the retinal field. Genetic screens to generate loss of function *dChx* phenotypes by P-element mediated mutagenesis are underway. The identification of *dChx* regulatory pathways is likely to lead to the recognition of homologous upstream and downstream components of the mammalian *Chx10* developmental network.

**Generation of mouse models of human congenital malformation syndromes using ENU mutagenesis. B.J.**

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Mouse models of congenital malformation syndromes can be useful both for understanding mammalian development and for determining the molecular basis of birth defects. We are screening embryos derived from mice mutagenized with N-ethyl-N-nitrosourea (ENU) for recessive phenotypes similar to human congenital defects. 3rd generation progeny are evaluated for developmental anomalies at embryonic d18.5; we thus identify defects in organogenesis that are consistent with survival *in utero* to late gestation, but which may cause post-natal lethality. Affected progeny are used directly for genetic mapping utilizing our previously described method of interval haplotype analysis. With this strategy we are able to localize the mutation to a single linkage group by genotyping 8-10 mice.

The main component of the mutation screen is an inspection of major organs after embryo dissection, followed by a more detailed analysis for craniofacial, kidney, brain and skeletal abnormalities. In our first study we identified 15 monogenic mutants in 54 families. The addition of a mapping component has proven extremely useful in prioritizing our analyses and expediting characterization. We have mapped 7 loci, identified the causal gene for 2 of the mutants obtained, and demonstrated by exclusion that a number of the unmapped mutations are not new alleles of known mutants with similar phenotypes. The spectrum of abnormalities found to date is remarkably varied, and many of these disorders are similar to human malformation syndromes. For example, we have generated models of asphyxiating thoracic dystrophy, Robin sequence, congenital diaphragmatic defect, non-syndromic cleft palate, polycystic kidney disease, and congenital heart defects. Our results demonstrate that a recessive screen can be extremely productive for the purpose of generating abnormal developmental phenotypes and can be readily managed even in a small laboratory.

**Single tube gene-specific expression analysis by high primer density multiplex reverse transcription.** *R.C. Clipsham, E.R.B. McCabe.* Dept Pediatrics, UCLA Sch Med, Los Angeles, CA.

Molecular genetics is rapidly moving from simple identification of a gene of interest to characterization of gene products as components in complex networks. Critical tools for gene product analysis require a rapid method for evaluation of contextual expression. Here, we describe a robust, high primer density, multiplex reverse transcription (RT)-polymerase chain reaction (PCR) technique capable of analyzing for the presence of numerous transcripts in a single tube. This assay substantially increases the total number of different cDNAs for amplification beyond previously published techniques. Our approach simultaneously eliminates RNA quality control issues for samples run in parallel while improving efficiency in the use of time and materials. This assay is designed for broad applicability, employs modifications of commercially available components and allows more than 25 independently selected gene-specific primers to be used simultaneously. Our protocol allows multiplexed primers to behave similarly to uniplex RT reactions, while avoiding potential interference between gene-specific and /or nonspecific primers during annealing and reverse transcription. Expression of putatively networked homologous transcripts was analyzed in multiple cell lines and tissues from mouse and human to validate the technique.

**Regulation of *Cbfa1/Runx2* action during osteoblast development by a *cbfa1*-interacting protein, *Cip*.** G. Zhou, P. Hermanns, Y. Chen, B. Lee. Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

CBFA1/RUNX2 is one of three vertebrate runt family transcription factors which have in common amino acid motif that mediates both DNA binding and protein-protein interaction. In transfection studies, *Cbfa1* can directly activate transcription of several bone-specific genes. *Cbfa1* null mice have complete absence of osteoblast and bone but an intact cartilagenous skeleton. CBFA1's requirement for osteoblast differentiation is supported by the finding of loss of function mutations in the human skeletal dysplasia-cleidocranial dysplasia (CCD). While *Cbfa1* is essential for osteoblast differentiation, additional factors are likely required to maintain the differentiation program. To identify potential CBFA1-interacting proteins, we screened a human osteosarcoma cDNA library using a yeast two-hybrid approach and isolated a 500-bp partial cDNA which we named CIP. Sequence analysis revealed homology to several anonymous human and mouse ESTs and Northern analysis and in situ hybridization on mouse E15.5 embryos revealed ubiquitous expression. The full-length cDNA was obtained by RT-PCR. It encodes a 297 amino acid polypeptide with no homology to known proteins. It has a multidomain structure with several protein-protein interaction domains and a nuclear localization signal. In transient transfections CIP down-regulated target gene transactivation by CBFA1 by 50%. FISH and radiation hybrid mapping showed that CIP localizes to chromosome 8q22 syntenic with a hypothesized second CCD-like locus. CIP mutation analysis in patients with CCD-like phenotypes who do not have mutations in CBFA1 revealed several potential polymorphism in its regulatory elements. We would predict that upregulation of *Cip*, a putative repressor of *Cbfa1*, would cause loss of *Cbfa1* transactivation and hence a CCD-like phenotype. To elucidate the potential in vivo effect of *Cip* on skeletogenesis, we are generating *Cip* null mice and transgenic mice overexpressing *Cip* in osteoblasts.

**A search for down-regulated genes in a DiGeorge syndrome mouse model.** *T. Pramparo, A. Baldini, E. Lindsay.* Pediatrics/cardiology, Baylor College of Medicine, Houston, TX.

Df1/+ mice carry a 1 Mb heterozygous chromosome deletion, which includes most of the murine homologs of genes deleted in DiGeorge syndrome. The deletion is associated with cardiovascular, thymic and parathyroid abnormalities. Our goal is to identify genes that are down-regulated in this model, as they may be downstream effectors of the haploinsufficient gene, *Tbx1*. To achieve this goal, we have used a commercially available cDNA subtraction kit (Clontech). The kit is based around a technique of subtractive hybridization that allows one to compare two different mRNA populations, in this case Df1/+ and wildtype, and thereby to obtain cDNA clones that represent genes that are expressed in one population but not in the other. At E10.5, *Tbx1* is highly expressed in the pharyngeal arch region, we therefore used as starting material for the cDNA subtraction, RNA that was extracted from segments of embryos encompassing the entire pharyngeal arch region and the heart. Following cDNA subtraction, we obtained 25 clones that were confirmed to be down-regulated in Df1/+ embryos. Sequencing of these clones revealed that two were transcription factors of the Forkhead and Sox families and a third clone that was a Rho family member. Of the remaining 22 clones, 7 were enzymes, 11 were other non-enzyme proteins and 4 were unknown sequences. We are currently confirming down-regulation of the transcription and Rho-related clones by in situ hybridization on Df1/+ and wild-type embryos. Moreover, we approached this goal by screening an oligonucleotide array (Affymetrix) of 6,000 full-length mouse genes, plus 6,000 clustered ESTs using cRNA probes made from the same starting material of the cDNA subtraction. This analysis identified several differentially expressed genes of interest that may be involved in the *Tbx1* pathway. Overall, our results demonstrate the feasibility of cDNA subtraction as a means to identify down-regulated genes in a haploinsufficiency model. Our preliminary results show that some candidate downstream targets of *Tbx1* may be transcription factors involved in the mesenchymal specification and differentiation. Supported by: A.H.A. 0060099Y to EAL.

**Developmental candidates for ocular colobomas: VAX1, VAX2 and SHH.** *L.A. Schimmenti<sup>1</sup>, J.D. Karkera<sup>2</sup>, J. dela Cruz<sup>2</sup>, H. Tran<sup>1</sup>, G.S. Manligas<sup>1</sup>, E. Roessler<sup>2</sup>, M. Muenke<sup>2</sup>.* 1) Dept Human Genetics, Pediatrics and the Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA; 2) Medical Genetics Branch, NIH/NHGRI, Bethesda, MD.

Ocular colobomas represent a heterogeneous phenotype caused by maldevelopment of the optic cup and stalk. It is estimated that 10% of childhood blindness is caused by colobomatous eye malformations. Ocular colobomas may present as abnormalities of the iris, retina or optic nerve and may include severe phenotypes such as microphthalmia and anophthalmia. We have previously demonstrated that autosomal dominant mutations in *PAX2*, a transcription factor expressed in the developing optic cup and stalk, cause optic nerve colobomas as part of the renal-coloboma syndrome. As most individuals with colobomas are not part of large kindreds, but rather isolated probands, we could not perform linkage analysis. We therefore hypothesized that genes expressed in the developing optic cup and stalk or genes that control *PAX2* expression would represent developmental candidate genes for ocular colobomas. *VAX1* is a transcription factor expressed in the optic cup and stalk in a spatial and temporal pattern similar to *PAX2*. *Vax1* knockout mice exhibit colobomas. *VAX2*, a related transcription factor, is expressed primarily in the optic cup along the optic fissure. *SHH*, a secreted factor, controls *PAX2* expression in the developing eye. Patients with holoprosencephaly (HPE) have been described with coloboma and coloboma has been observed in family members of HPE patients. We therefore analyzed *VAX1*, *VAX2* and *SHH* genes for mutations in genomic DNA isolated from 52 probands with ocular colobomas. The majority of patients carried the diagnosis of non-syndromic colobomas and microphthalmia. CHARGE syndrome was diagnosed in 4 individuals. We performed single stranded conformational polymorphism analysis, dHPLC or direct sequencing on amplimers of the coding exons of *VAX1*, *VAX2* and *SHH*. Screening of *VAX1* and *VAX2* has not identified causative mutations in patients with ocular colobomas. *SHH* analysis is still in progress.



**Further Characterization of the X-linked Dominant, Male Lethal Bare Patches Mutation.** *G.E. Herman<sup>1</sup>, X. Wang<sup>1</sup>, M. Lucas<sup>1</sup>, Q. Ma<sup>1</sup>, J. Peters<sup>2</sup>, B. Cattanach<sup>2</sup>, C. Pierson<sup>3</sup>, M. Bard<sup>3</sup>, R.I. Kelley<sup>4</sup>*. 1) Dept of Pediatrics, Children's Research Inst., Columbus, OH; 2) Mammalian Genetics Unit, Harwell, UK; 3) Dept of Biology, Indiana-Purdue University, Indianapolis, IN; 4) Kennedy Krieger Inst., Baltimore, MD.

The X-linked mouse mutation bare patches (Bpa) and most cases of human CHILD syndrome result from mutations in *Nsdhl* (for NADH steroid dehydrogenase-like). *Nsdhl* is involved in the removal of C-4 methyl groups in the cholesterol biosynthetic pathway. While the features in surviving affected human and murine females have many similarities, the striking unilateral skin and skeletal phenotype found in CHILD syndrome is not seen in the mouse. We have now detected missense mutations V53D and A94T in conserved amino acids in two additional Bpa alleles. Functional complementation assays of these mutations in yeast demonstrate their significance. The latter mutation is identical to one identified in 2 unrelated CHILD patients (*Amer. J. Med. Genet.* 90:339-346, 2000). Differences in the phenotype between Bpa mice and CHILD syndrome are unlikely to be explained on the basis of different types or sites of mutations. Male embryos for several Bpa alleles die during midgestation, and cholesterol levels in affected male embryos at the time of death are normal. Placentas from affected male embryos at day 10.5 are smaller than those of normal littermates ( $p < 0.001$ ) and the labyrinthine layer of the placenta appears thinner and disorganized. There are also statistically significant differences in placental thickness between affected male and affected female placentas. Most cells in the female rodent placenta undergo preferential inactivation of the paternal X Chr. We believe that mesodermally derived cells that undergo random X-inactivation are responsible for the male lethality. Finally, we have been unsuccessful in attempts to generate transgenic mice that overexpress *Nsdhl*. Preliminary data from experiments in which a tyrosinase minigene was coinjected with an *Nsdhl* cDNA suggest that such overexpression may be lethal during embryogenesis. A possible explanation is abnormal feedback regulation of isoprenoid synthesis.

**X-linked gene expression and methylation in Rett syndrome: role of MeCP2 in epigenetic control of X chromosome inactivation.** *L. Carrel, B.P. Chadwick, K.C. Trevarthen.* Dept. Genetics, Case Western Reserve Univ., Cleveland, OH.

Rett syndrome (RTT) is caused by mutations in the X-linked gene MECP2 that encodes a methyl-cytosine binding protein involved in transcriptional repression via a histone deacetylase complex. This suggests that the RTT phenotype results from at least partial derepression of normally silenced genes. Target genes remain unknown, but plausible candidates in RTT females include any of the several thousand genes on the X that are normally epigenetically silenced by X inactivation. X inactivation patterns in most RTT females are random, establishing that MeCP2 is not essential for X inactivation. However, multiple methyl-binding proteins have been identified and, therefore, MeCP2 may be necessary for silencing only a subset of X-linked genes.

To determine whether X-linked gene expression or other epigenetic features of X inactivation are altered in RTT individuals, lymphoblast or fibroblast cell lines from 5 RTT females were subcloned to isolate lines that carry the mutant MECP2 allele on the active X. In each case, clonality was established by monoallelic expression of a transcribed XIST polymorphism and/or by methylation analysis at the Androgen Receptor (AR) locus. Additionally, correct clones exclusively expressed the mutant MECP2 transcript. The XIST gene, normally expressed only from the inactive X, and 10 X-linked genes normally silenced on the inactive X were then tested using transcribed polymorphisms to distinguish active and inactive X expression. All 28 informative loci analyzed (3-8 genes/cell line) showed complete monoallelic expression, indicating that their expression is not altered in the RTT lines. Intriguingly, one of three RTT lymphoblasts tested showed aberrant methylation; 5 subclones from this line were completely unmethylated at AR on both the active and inactive X. These results suggest that MeCP2 likely has a role in X inactivation, yet because of functional redundancy, proper inactivation for X-linked genes can occur in the absence of MeCP2. Additionally, these data caution that methylation-based assays may not accurately predict X inactivation ratios in all RTT females.

**DsRNAi and overexpression of the *C.elegans* orthologue of MADSO1, a novel HC21 gene, suggest an important role in embryonic development and adult morphogenesis.** *O. Menzel*<sup>1</sup>, *T. Vellai*<sup>2</sup>, *K. Takacs-Vellai*<sup>2</sup>, *A. Reymond*<sup>1</sup>, *F. Mueller*<sup>2</sup>, *S.E Antonarakis*<sup>1</sup>, *M. Guipponi*<sup>1</sup>. 1) Division of Medical Genetics, University of Geneva, CMU, Switzerland; 2) University of Fribourg, Institute of Zoology, Perolles, Switzerland.

Down syndrome (DS), the most common cause of mental retardation, is caused by the presence of an extra copy of some but certainly not all the approximately 220 genes on human chromosome 21 (HC21). In order to identify those genes, we isolated a new transcript, MADSO1. This gene contains 9 exons and spans 24kb of genomic sequence on 21q22.3 between ADARB1 and COL18A1. Two ubiquitous mRNAs of 3.3 and 5kb respectively and a brain-specific transcript of 2.7kb are expressed. Transfection of tagged-MADSO1 protein revealed a perinuclear localization. Colocalization experiments showed a probable localization for MADSO1 protein within the Golgi apparatus. The human protein is highly conserved in *C.elegans* and in *Drosophila* (52% and 35% identical, respectively) and contains a MADS box domain. To investigate the function of MADSO1, we isolated the *C. elegans* orthologue and performed dsRNAi and overexpression experiments. DsRNAi was used to decrease the endogenous level of CeMADSO1 protein. 5-7 % of the progeny (F1) of the adults nematodes microinjected or fed (F0) with CeMADSO1 dsRNA showed embryonic arrest mainly after gastrulation and around morphogenesis. The majority of F1 adults exhibited a partial Egl phenotype (Egg-laying defective) and approximately 2% became extremely malformed with a highly deformed vulva region and gonadal structure. Overexpression analysis was used to determine the effect of an excess of CeMADSO1 protein. CeMADSO1 was ectopically overexpressed under the control of a *C.elegans* heat-shock promotor in all the tissues of adult *C.elegans*. Embryonic lethality was observed. These results indicate that CeMADSO1 is essential for the development and the morphogenesis of *C. elegans*. This study provides the first example of the use of *C. elegans* as a model to study the effects of silencing and overexpression of genes on HC21 that might be involved in Down syndrome.

**Microarray gene expression analyses in cells transfected with sense and antisense sequences of a novel activator of cyclin-dependent kinase (ACDK4).** *H. Zhao, C. Wang, P. Yang, C. Collins, S. Eckenrode, Q. Ruan, R. McIndoe, J. She.* Dept. of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL.

ACDK (activators of cyclin-dependent kinases) is a newly identified gene family encoding four individual proteins. Our functional study revealed that ACDK is a novel group of cell cycle regulatory proteins which can promote cell cycle progression by activating CDKs (cyclin-dependent kinases) to phosphorylate the retinoblastoma protein (pRB). In the present study, we established stable cell lines with both sense and antisense constructs in a pBIG2i inducible tet-on vector using ACDK4 coding sequence in HeLa cells. Genes with differential expressions between sense and antisense clones were analyzed by cDNA microarray technology. A total of 12288 human cDNA clones (from Research Genetics HTVV library) were analyzed in our study. RNAs from untransfected cells, sense and antisense cell clones were mixed as reference for normalization. Cy-dye labeled cDNA probes from 6 sense and 6 antisense clones (after induced 48hrs) were hybridized with the human cDNA clones. Discriminant and hierarchical clustering analyses showed that 37 genes were up-regulated in sense clones, and 58 genes were up-regulated in antisense clones. Database search revealed ten out of 37 genes are known genes for the sense clones, most of them are cell cycle related proteins, e.g. CCNK, PTMA, NEB, TGR, and PRP18 etc, which suggests that these genes are probably regulated by the ACDK proteins. While only four out of 58 genes are known genes for the antisense clones, most of them are ion channel proteins.

**Identification of proteins that interact with human diaphanous, DFNA1.** *A.B.S. Giersch<sup>1</sup>, C.L. Beattie<sup>2</sup>, P.L. Welch<sup>2</sup>, M-C. King<sup>2</sup>.* 1) Department of Pathology, Brigham and Women's Hospital, Boston, MA; 2) Departments of Medicine and Genomic Sciences, University of Washington, Seattle, WA.

DFNA1 is responsible for nonsyndromic sensorineural progressive hearing loss in a large Costa Rican kindred. Mutant DFNA1 is truncated 32 aa from the C-terminus of the 1251-aa wildtype protein. DFNA1 is a human homolog of *Drosophila* diaphanous, an essential mitotic gene and effector of the small GTPase Rho. Rho regulates actin cytoskeleton by shuttling between the GDP-bound inactive form and the GTP-bound active form. The active form causes reorganization of active stress fibers while inactivation leads to dissolution of these structures. As a formin homology (FH) protein, diaphanous contains the Rho-binding domain and three FH regions. Its FH1 domain interacts with profilin to induce actin polymerization. Also, the diaphanous C-terminus interacts with its N-terminal Rho-binding domain. Thus GTP-bound Rho may interact with diaphanous to expose FH domains and activate actin polymerization. Given that actin is the major component of the cytoskeleton of hair cells of the inner ear, we postulated that mutant diaphanous may disrupt Rho-mediated regulation of actin polymerization. In a yeast two-hybrid screen we identified three proteins that interact specifically with the C-terminus of human diaphanous: estrogen-related receptor alpha (ESRRA); ATP-binding cassette, sub-family D, member 4 (ABCD4); and protein phosphatase 2, regulatory subunit B (B56), alpha (PPP2R5A). ESRRA (11q12) is an orphan nuclear receptor homologous to the estrogen receptor that binds similar DNA target sites. However, ESRRA is not activated by classic estrogens and its target genes are not yet known. ABCD4 (14q24.3) is a novel human ABC-half-transporter. PP2A is a heterodimeric serine/threonine protein phosphatase implicated in cell growth and division, muscle contraction, and transcription, whose alpha subunit gene (1q41) encodes a cytoplasmic phosphoprotein. To determine if these proteins may play a role in hearing, we are testing their interaction with the human mutant DFNA1 in the two-hybrid system.

**Inner ear localization of mRNA and protein products of *COCH*, mutated in the sensorineural deafness and vestibular disorder, DFNA9.** *N.G. Robertson*<sup>1</sup>, *B.L. Resendes*<sup>1,3</sup>, *J.S. Lin*<sup>1</sup>, *C. Lee*<sup>1,3</sup>, *J.C. Aster*<sup>1,3</sup>, *J.C. Adams*<sup>3,4</sup>, *C.C. Morton*<sup>1,2,3</sup>. 1) Dept Pathology, Brigham & Women's Hosp, Boston, MA; 2) Dept Ob/Gyn, Brigham & Women's Hosp, Boston, MA; 3) Harvard Medical School, Boston, MA; 4) Dept Otology and Laryngology, Mass Eye and Ear Infirmary, Boston, MA.

Missense mutations in the *COCH* gene, which is expressed at high levels and preferentially in the inner ear, cause the autosomal dominant sensorineural deafness and vestibular disorder at the DFNA9 locus. By *in situ* hybridization of mouse and human inner ear sections, we find high-level expression of *COCH* mRNA in the fibrocytes of the spiral limbus and of the spiral ligament in the cochlea, and in the fibrocytes of the connective tissue stroma underlying the sensory epithelium of the crista ampullaris of the semicircular canals.

We also developed a polyclonal antibody against the human *COCH* protein product, cochlin. The antibody was raised against the N-terminal 135 amino acid residues of cochlin, corresponding to the *Limulus* factor C-homology (cochFCH) domain, which harbors all five known point mutations in DFNA9. On Western blots, anti-cochlin reacts with bacterially-expressed cochlin constructs and not with another control protein expressed in the same bacterial strain. Western blots also reveal high levels and different isoforms of cochlin in extracts of human fetal cochlea.

Immunohistochemistry performed with anti-cochlin shows staining predominantly in the regions of the fibrocytes of the spiral limbus and of the spiral ligament in mouse and human fetal and adult tissue sections. These sites correspond to those that express *COCH* mRNA as determined by *in situ* hybridization, and to the regions of the inner ear which show histological abnormalities in DFNA9. The fibrocytes expressing mRNA and protein products of *COCH* are the very cell types which are markedly absent in temporal bone sections of individuals affected with DFNA9, which also show a striking accumulation of a homogeneous eosinophilic acellular material replacing the normal fibrocytes.

**A single amino acid substitution in the FMR protein (I304N) results in reduced phosphorylation and an altered conformation.** *S.S. Ceman*<sup>1,2</sup>, *S. DiMarco*<sup>3</sup>, *S.T. Warren*<sup>1,2</sup>. 1) Howard Hughes Medical Institute; 2) Dept Genetics, Emory Univ, Atlanta, GA; 3) Boston University.

Fragile X syndrome is caused by the absence of the protein product of the FMR1 locus. An exception has been found in a severely affected individual who has normal levels of FMRP but with a single amino acid substitution at position 304 (I304N). The I304N protein has been extensively characterized and has many features distinguishing it from the wild type protein: it is not found associated with polysomes, forms a smaller mRNP particle, has altered RNA binding, shuttles more rapidly through the nucleus and is unable to form homodimers. We show here that unlike normal FMRP, which is serine-phosphorylated in a number of different cell lines labeled with [32P] ortho-phosphate and isolated by immunoprecipitation, the I304N protein is poorly phosphorylated. Phosphorylation of wild type FMRP is inhibited by 5,6 dichloro 1-B-D ribofuranosyl benzimidazole, which inhibits a limited number of kinases, including CK2. CK2 does co-immunoprecipitate with FMRP suggesting that it may be a substrate. In addition, we found that the I304N protein is not recognized efficiently by a newly developed monoclonal antibody, 6G2, which readily recognizes wild type FMRP. The epitope recognized by mAb 6G2 is N-terminal to amino acid 304 at peptide 131-142. Because this peptide contains no serines, the loss of reactivity of 6G2 may be due to an altered conformation of the I304N protein.

**Intracellular distribution of lysosomal sialidase is controlled by the internalization signal in its cytoplasmic tail.**

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Sialidase (neuraminidase), encoded by the *neu-1* gene in the MHC locus catalyzes the intralysosomal degradation of sialylated glycoconjugates. Inherited deficiency of sialidase results in sialidosis or galactosialidosis, both severe metabolic disorders associated with lysosomal storage of oligosaccharides and glycopeptides. Sialidase also plays an important role in cellular signalling and is specifically required for the production of cytokine IL-4 by activated T lymphocytes. In these cells, *neu-1*-encoded sialidase activity is increased on the cell surface, suggesting that a specific mechanism regulates sorting of this enzyme to the plasma membrane. We investigated that mechanism by first showing that sialidase contains the internalization signal found in lysosomal membrane proteins targeted to endosomes via clathrin-coated pits. The signal consists of a C-terminal tetrapeptide 412YGTL415, with Tyr412 and Leu415 essential for endocytosis of the enzyme. We further demonstrated that redistribution of sialidase from lysosomes to the cell surface of activated lymphocytes is accompanied by increased reactivity of the enzyme with anti-phosphotyrosine antibodies. We speculate that phosphorylation of Tyr412 results in inhibition of sialidase internalization in activated lymphocytes.



**Protein tyrosine phosphatase families resulted from gene duplication, fusion, horizontal transfer and circular permutation.** *J.-F. Huang, C.-Q. Liu.* Lab. Cell. & Mol. Evol., Kunming Institute of Zoology, Kunming, Yunnan, P. R. China.

Protein tyrosine phosphatases (PTPs) play a key role during cell signal transduction, which contain three families, low-molecular-weight phosphotyrosine phosphatases (lmwPTPs), higher-molecular-weight phosphotyrosine phosphatases (hmwPTPs) and dual specificity phosphatases (DSPs), and lmwPTP family is considered as an example of convergent evolution with hmwPTP and DSP families in early studies. In this work, we have searched 98 genomes/NR database and compared PTP sequences, structures and functions, the results indicate that PTP gene copies in these three families are increased from archaeotes/virus to eubacteria and to eukaryotes, and the gene encoding lmwPTP can be found in archaeotes, virus, eubacteria, and eukaryotes; but those encoding hmwPTP and DSP are not found in archaeotes. Sequence analysis and structure/function comparison show that these three families have similar structure, function, catalytic mechanism and a common functional motif, but low sequence identities, a different order of active site in sequence (or circular permutation) between lmwPTP and hmwPTP/DSP families, and the difference in substrate specificity between lmwPTP and DSP. On the other hand, the part of N-terminal in mRNA capping enzyme is a domain with similar DSP function, which cannot be found in bacteria, but in virus and eukaryotes. Thus, the results suggest that hmwPTP and DSP families are evolved from lmwPTP gene, the circular permutation in sequence of PTPs is resulted from the ancestral lmwPTP gene duplication, fusion and different reading, and which could be one of the reasons caused the different substrate specificities between lmwPTPs and DSPs. The DSP-like domain in mRNA capping enzyme might come from DSP in virus by horizontal gene transfer.

**Interspecific Comparisons of 35 Nuclear Receptor Superfamily Members Show the *NROB* Subfamily to be Permissive for Sequence Variability.** *M. Patel*<sup>1</sup>, *G.B. Golding*<sup>2</sup>, *A.P. Arnold*<sup>1</sup>, *J.S. Sinsheimer*<sup>3,4</sup>, *E.R.B. McCabe*<sup>5</sup>. 1) Dept. of Physiological Sciences, UCLA, Los Angeles, CA; 2) Dept. of Biology, McMaster University, Hamilton, ON, Canada; 3) Dept. of Biomathematics, UCLA, Los Angeles, CA; 4) Dept. of Human Genetics, UCLA, Los Angeles, CA; 5) Dept. of Pediatrics, Mattel Children's Hospital, UCLA School of Medicine, Los Angeles, CA.

We have previously shown that in closely related primate species the X-linked gene, *NROB1* encoding the nuclear receptor (NR) superfamily member DAX1, is predisposed to amino acid-changing substitutions similarly to *SRY* and greater than *SOX9*. The purpose of the current investigation was to compare *NROB1* sequence changes during evolution with 34 other members of the NR superfamily from all seven subfamilies, including the other *NROB* subfamily member, *NROB2*, which codes for SHP. We compared the coding sequences of these genes 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. Codon usage and GC content at silent sites of synonymous codons (GC3s) were compared. We also performed correspondence analysis on codon usage for these genes in human and mouse. We found that codon usage correlated with GC3 content, superfamily, subfamily and ligand binding group. Among the genes we compared between human and mouse, *NROB1* and *NROB2* had the highest Ka values ( $0.221 \pm 0.016$  and  $0.117 \pm 0.015$ , respectively) and Ka/Ks ratios (32.03 and 18.88, respectively). We conclude that the *NROB* subfamily is permissive for sequence variability. We speculate that the sequence variability of the *NROB* subfamily members may reflect their evolutionary history as well as functional properties of their protein products.

**A novel human MOB gene encodes a member of novel family of transmembrane proteins.** *I.P. Vladychenskaya, L.V. Dergunova, S.A. Limborska.* Dept Molecular Basis, Inst Molecular Genetics, Moscow, Russia.

As the result of the differential hybridization of the human medulla oblongata cDNA library the 1,6 kb brainspecific clone Hmob33 was obtained (Acc. No. Y14155). Hmob33 sequence was searched with BLAST against the human cDNA and genomic sequences. The data revealed the highest similarity between 5'-end of Hmob33 and 3'-end of AK026683, which is a 2,2 kb anonymous cDNA clone from the human lung. These overlapping clones were used for constructing the 3,2 kb HMOB33 contig. HMOB33 contig has appeared to include the coding region flanked with the untranslated regions. It probably represents one of the mRNA transcription variants of some unknown gene named MOB gene. The results of the Northern blot analysis confirmed the predicted size and brainspecific expression pattern of the corresponding transcript. BLAST analysis of the human genomic sequences revealed the high similarity of the whole HMOB33 contig with two different genomic clones of the human chromosome 10. The alignment of HMOB33 contig relative to these clones breaks the contig into the fragments which have the identical patterns of distribution along both genomic clones thus representing the presumed exon-intron structure (7 exons and 6 introns spanning the region more than 155,5 kb) for the proposed MOB gene. The hypothetical product of MOB gene is assigned to be a five pass transmembrane protein of 413 amino acids with its N-terminus oriented into the cytoplasm and bearing the protein-protein interaction module. BLAST-analysis has revealed a number of anonymous putative proteins similar to the Mob protein coming from mouse, fruit fly and nematoda. The proposed exon-intron structure of two genes encoding two proteins from mouse is identical for that for the human MOB gene. BLAST search of the corresponding mouse mRNA sequences through the human genomic sequences database proposed the existence of the human gene related to MOB gene on the human chromosome 4. All the putative proteins coming from the named organisms are predicted to be the transmembrane molecules. These data argues for the existing of thus far unknown gene family of transmembrane proteins.

**Genomic organization and alternative transcription of a novel human SAM-dependent methyltransferase gene on chromosome 2p21-p22.** *Y. Zhang<sup>1</sup>, M.C. Gorry<sup>1</sup>, P.S. Hart<sup>2</sup>, M.J. Pettenati<sup>3</sup>, X. Lu<sup>1</sup>, L. Wang<sup>1</sup>, J.J. Marks<sup>1</sup>, B. Suppe<sup>1</sup>, T.C. Hart<sup>1,2</sup>.* 1) Oral Biol & Medicine, Univ Pittsburgh Sch Dental Med, Pittsburgh, PA; 2) Genetics, Univ Pittsburgh Sch Public Health; 3) Pediatrics, Wake Forest University Sch Medicine.

Gene identification and characterization of genomic structure in the post-genome-era increasingly involves the integration of bioinformational and laboratory methodologies. Using this strategy, we have identified a novel human methyltransferase gene (288L6 methyltransferase - 288L6 MTase). This gene contains the highly conserved methyltransferase domain characteristic of SAM-dependent methyltransferases. We localized this gene to chromosome 2p21-p22 by FISH, and sublocalized to BAC288L6 by homologous search. Computational analysis of aligned ESTs identified 11 exons in the hypothetical 288L6 MTase gene spanning 46 kb sequences. Results of RACE analysis in placenta suggest there are multiple transcripts, with three predominant forms of transcript I, II, and III. Two alternative polyadenylation sites are present in this gene. Sequencing of these three cognate transcripts demonstrates heterogeneity at the 5' untranslated region. Alternative transcription and tissue specific expression of this gene are detected by Northern blot analysis. The 288L6 MTase gene is expressed in a variety of tissues including brain, heart, colon, thymus, spleen, kidney, liver, small intestine, placenta, and lung. Open reading frame analyses of the three alternative transcripts identified a shared coding region spanning exons 7-11. This ORF consists of 732 nucleotides encoding a putative 244 amino acid protein. Bioinformational searches of both the 288L6 MTase DNA and the putative protein product identified three methyltransferase motifs conserved across many prokaryotic and eukaryotic species. The genomic structure and alternative transcripts of 288L6 MTase gene were determined. Since enzymatic methylation is important in cellular processes, including differentiation, growth, gene regulation, embryonic development, and oncogenesis, it will be important to functionally characterize the 288L6 MTase. (supported in part by NIDCR R01-DE12771).

**Identification of Human FEM1A, the homologue of a *C. elegans* sex-differentiation gene.** *D. Krakow*<sup>1,2</sup>, *E. Sebald*<sup>2</sup>, *L.M. King*<sup>2</sup>, *D.H. Cohn*<sup>2,3,4</sup>. 1) Dept OB/GYN Cedars-Sinai Medical Ctr and UCLA School of Medicine; 2) Medical Genetics-Birth Defects Center/Ahmanson Dept of Pediatrics; 3) Dept of Human Genetics, UCLA School of Medicine; 4) Dept of Pediatrics, UCLA School of Medicine, LA CA.

We report the isolation, genomic structure, chromosomal location, and expression pattern of the FEM1A gene, the human homolog of the *C.elegans* fem-1 and mouse Fem1a genes. The coding sequence is 1851 bp and encodes a 617 amino acid protein. The human FEM1A protein has 65% identity with the mouse Fem1a protein and 34% identity with the *C.elegans* fem-1 protein, indicating conservation of this gene. The N-terminal region of the encoded protein contains 6 ANK repeat elements, a motif found in signaling and transcriptional regulatory molecules such as Notch and glp1. The gene was highly expressed in human skeletal muscle and cardiac tissue, and was expressed at lower levels in multiple tissues, including cartilage. FEM1A was localized to chromosome 5q23.1, a region of conserved synteny with a portion of mouse chromosome 17 that contains Fem1a. In *C.elegans*, fem-1 is involved in a pathway necessary for sex-determination. The identification of a human homolog of this conserved gene suggests a potential role for this sex-determining molecule in humans.

**Isolation and characterization of the ALX4 promotor region.** *W. Wuyts, W. Van Hul.* Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.

The Alx4 gene is a homeodomain transcription factor related to the *Drosophila aristaless* gene. Expression studies have shown that Alx4 is expressed in mesenchymal cells at several sites in the embryo, including the craniofacial mesenchyme. Alx4 KO mouse show preaxial polydactyly, ventral body wall defects and a delay in the formation of the parietal bone of the skull. Previously, we isolated the human ALX4 gene out of a region which is deleted in patients suffering from the Proximal 11p Deletion Syndrome (P11pDS) and showed that in humans inactivating mutations in ALX4 cause skull ossification defects (Foramina Parietalia Per magna; FPP). To identify potential regulators of ALX4 expression we isolated and analyzed the 5' region flanking the ALX4 gene. By reverse transcriptase mapping the ALX4 transcription start site (TSS) was mapped in a region located approximately 700 bp from the start codon and further fine mapping of the TSS was obtained by 5' RACE experiments. Several fragments surrounding the TSS were generated by PCR and exonuclease/S1 treatment and cloned in the pGL3 Luciferase vector. By a dual Luciferase reporter assay, promotor activity of the various constructs was analyzed in two osteosarcoma cell lines to identify the minimal promotor sequences and potential positive and negative regulating regions.

**Promoter analysis of Kv8.1 revealed the essential element for Kv8.1 expression.** *M. Ebihara, H. Ohba, T. Yoshikawa.* Lab Molec Psychiatry, Brain Sci Inst, Riken, Saitama, Japan.

It has been reported that there are more than seventy potassium channel genes in human. Some of these are associated with human diseases, such as long QT syndrome, episodic ataxia and epilepsy. It has been suggested that mutations of KCNQ channel genes cause benign familial neonatal convulsions (BFNC) and other epilepsies are strongly associated with channel proteins, i.e. potassium channel, sodium channel and nicotinic cholinergic receptor. In 1991, Yasuda et al. have reported benign adult familial myoclonic epilepsy (BAFME) among Japanese population. Mikami et al. have reported the gene responsible for BAFME is mapped on the distal portion of 8q (8q23.3-24.11). We have isolated and determined whole genomic structure of Kv8.1 potassium channel gene that is mapped on human chromosome 8q23-24. In the previous report, it has been reported that Kv8.1 has 3 exons, but our analysis of genomic structure has revealed the Kv8.1 has 4 exons indicating the existence of an additional 5' exon has been found. We also detected its promoter elements in upstream region. Series of deleted promoter mutants were constructed to determine the minimum region of Kv8.1 promoter. Luciferase assay showed that 130 bp upstream region is required for full activation of Kv8.1 expression. Two sp1 elements and some other elements have been found in this region suggesting they are essential for Kv8.1 expression. Here we show Kv8.1 genomic structure and its promoter region as a candidate gene for BAFME.

**Different mutations in the spastin gene result in distinct electrophysiological phenotypes in patients with hereditary spastic paraplegia type 4 (SPG4).** *D. Boensch<sup>1,2</sup>, A. Schwindt<sup>1,3</sup>, P. Navratil<sup>1,3</sup>, D. Palm<sup>3</sup>, S. Klimpe<sup>1</sup>, J. Hazan<sup>4</sup>, C. Weiller<sup>5</sup>, T. Deufel<sup>1</sup>, J. Liepert<sup>5</sup>.* 1) Institut fuer Klinische Chemie, Friedrich-Schiller Universitaet, Jena, Germany; 2) Klinik fr Neurologie, Friedrich-Schiller Universitaet, Jena, Germany; 3) Abteilung fr Neuropaediatric, Kinderklinik der Universitaet Muenster; 4) Genoscope, 2 rue Gaston Cremieux, 91000 Evry; 5) Klinik fr Neurologie, Universitaet Hamburg.

Hereditary spastic paraparesis (HSP) denotes a heterogeneous group of inherited neurological disorders with progressive lower limb spasticity; pedigrees have been described with autosomal dominant, autosomal recessive and X-linked inheritance. Pure and complicated forms of HSP exist. Even in patients within the same pedigree, however, clinical presentation may vary to a large degree; similarly, electrophysiological tests show variable results: transcranial magnetic stimulation (TMS) studies have demonstrated that central motor conduction times (CMCT) to lower extremities are delayed in a majority but not all patients; CMCTs to upper extremities are usually normal. A large proportion of autosomal dominant cases of HSP have been found to belong to HSP type 4 on chromosome 2; the gene, spastin, was recently cloned and a variety of mutations have been identified in different pedigrees. We report on 2 families with pure HSP caused by different mutations in the spastin gene. Clinically, affected members in both pedigrees present with a disease that is very similar with respect to age of onset, progression and severity of symptoms. With the aim to reveal subtle phenotypic differences that might reflect different molecular changes in the two pedigrees, the motor system of both clinically affected and clinically unaffected subjects was evaluated using transcranial magnetic stimulation. Our results show differences in several categories depending on the type of mutation present; this finding provides, for the first time, a phenotypic correlate of a given genetic change in this gene with its, so far, largely unknown function.



Program Nr: 979 from the 2001 ASHG Annual Meeting

**A Resource for Models of Human Disease.** *J.M. Fuller, S.F. Rockwood, L.E. Mobraaten, M.T. Davisson.* Induced Mutant Resource, The Jackson Laboratory, Bar Harbor, ME.

Mouse models have become a principle tool for use in dissecting disease pathways and target drug development. The recent availability of mouse and human genome sequence information will only promote the use of the mouse models in a wide range of human disease-related applications. In an effort to support this trend, the Induced Mutant Resource (IMR) at The Jackson Laboratory makes available over 600 genetically engineered mouse models. Created in 1992, the IMR serves as a centralized facility to collect, cryopreserve and distribute induced mutant mice to the scientific community. Since its inception, the IMR has accepted over 800 different induced mutant mouse strains, distributing over 100,000 mice a year to the scientific community. Current growth in the IMR collection is approximately 70-80 new strains each year, offering new mouse models in many areas including cancer, diabetes, neurobiology, development and immunology. Included in the resource collection are recently accepted models for mucopolysaccharidosis type I (MPS I), thrombosis, von Hippel-Lindau (VHL) disease and Batten Disease (juvenile neuronal ceroid lipofuscinoses-JNCL). An on-line resource is available allowing researchers to retrieve information related to the mutant strains maintained in the IMR. Mutant strain records in the IMR database include brief phenotype descriptions, strain construction and husbandry information and a list 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. The IMR database can be accessed at URL: <http://www.jax.org/resources/documents/imr/>. The Induced Mutant Resource is supported by the National Center for Research Resources (RR09781), The National Institute for Allergy and Infectious Disease and The Howard Hughes Medical Institute.

**Direct analysis of MECP2 inactivation status in patients with Rett syndrome.** *I. Kondo<sup>1</sup>, R. Morishita<sup>1</sup>, T. Matsuishi<sup>2</sup>, Y. Yamashita<sup>2</sup>, T. Fukuda<sup>1</sup>, H. Yamagata<sup>1</sup>.* 1) Dept. of Hygiene, Ehime Univ. School of Med., Ehime, Japan; 2) Dept. of Pediatrics, Kurume Univ. School of Med., Fukuoka, Japan.

Rett syndrome (RTT) is an X-linked dominant disorder characterized by impaired neurodevelopmental system. Most patients with RTT have DNA mutations in a gene for methyl-CpG binding protein 2 (MECP2) mapped to Xq28. Over 120 mutations in MECP2 have been reported in patients with RTT and the genotype-phenotype correlation has been studied, but not established, because clinical phenotypes might be modified by the X-chromosome inactivation status. Most patients with RTT had random X-chromosome inactivation pattern using an androgen receptor (AR) polymorphism, but their healthy mothers with the same mutations had skewed X-chromosome inactivation. The X-chromosome inactivation has not always influenced on clinical severity in sporadic patients with RTT. The reason might be that the X inactivation status has been studied by AR polymorphism. We have detected a parental origin of DNA mutations of MECP2 in 50 sporadic patients with RTT and paternal MECP2 mutations were observed in 45 cases (90%). Then, to establish the genotype-phenotype correlation in RTT patients, we have directly analyzed MECP2 inactivation status by PCR amplification of HpaII digested DNA using allele specific primers and restriction enzymes. Most sporadic patients with RTT have random MECP2 inactivation patterns. However, a patient with the skewed expression of P152R mutation allele (80%) was more severely affected compared to patients with the random inactivation patterns of P152R mutations. On the other hand, a patient with R133C who had less expression of mutated allele had milder clinical phenotype. Then, the direct inactivation analysis of MECP2 is very useful to study the skewed gene expression status in patients with RTT.

**The novel association of SMN protein with a ribonucleoprotein complex containing two major nucleolar proteins is altered in Spinal Muscular Atrophy.** *S. Lefebvre, P. Burlet, L. Viollet, S. Bertrand, C. Huber, C. Belser, E. Vial, A. Munnich.* INSERM U-393, Hopital des Enfants Malades, Paris, France.

Spinal muscular atrophy (SMA) is caused by the loss of functional survival motor neuron (SMN) protein. This protein is part of a multifunctional complex involved in the assembly/regeneration of spliceosomal small nuclear ribonucleoproteins (snRNPs) and in the assembly of the RNA polymerase II machinery. Yet, which SMN-dependent function is responsible for the disease remains unclear. The SMN protein complex is detected in the cytoplasm and in the nuclear gems colocalized with coiled bodies often found at the periphery of the nucleolus. Here, we show that the SMA frameshift 472del5 mutation encodes a mutant SMN protein found into the nucleolus and/or the nucleoplasm of transfected COS cell cultures. It is the first SMA mutant to display an intranucleolar distribution. Co-immunoprecipitation studies carried out on untransfected cell nuclear extracts reveal that the normal SMN protein is associated with RNP complexes containing two major non-ribosomal nucleolar proteins and the association is mediated, at least, by RNA moieties. Most interestingly, the association is disrupted in fibroblast cell culture derived from an SMA type I patient (with an homozygous SMN1 gene deletion), showing a close correlation between the marked reduction of SMN protein level and defects in RNP complex assembly and/or stability. Our findings support the view that SMN protein is involved in different aspects of RNA processing and the identification of proteins in SMN-associated RNPs should help in understanding the pathogenesis of SMA.

**Identification of the proteins in neuroblastoma that were affected by overexpression of parkin.** *T. Kawamura*<sup>1</sup>, *A. Takayanagi*<sup>1</sup>, *S. Asakawa*<sup>1</sup>, *S. Minoshima*<sup>1</sup>, *Y. Mizuno*<sup>2</sup>, *N. Shimizu*<sup>1</sup>. 1) Department of Molecular Biology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan; 2) Department of Neurology, Juntendo University School of Medicine, Bunkyo-ku, Tokyo, Japan.

*PARKIN* was discovered as a pathogenic gene of the autosomal recessive juvenile parkinsonism (AR-JP). The parkin protein possesses ubiquitin-like domain and ring-finger motif as well as several phosphorylation sites. Recently, we found parkin is involved in protein degradation pathway by playing a role as an ubiquitin-protein ligase in the dopaminergic cells in substantia nigra. We postulated the substrate(s) for parkin may be accumulated in the pathogenesis of AR-JP. Patients of AR-JP lack Lewy body, the characteristic protein aggregates seen in sporadic Parkinsons disease. Parkin was found in Lewy body in substantia nigra of sporadic Parkinsons disease. Here, we attempted to identify parkin-associated proteins by comparing the expression patterns of cellular proteins in dopaminergic neuroblastoma cell line SH-SY5Y with and without infection of recombinant Adenovirus with parkin cDNA. Effects of a proteasome inhibitor and a differentiating agent were also assessed. Identification of the proteins was performed by two-dimensional polyacrylamide gel electrophoresis followed by tandem mass spectrometry (MS/MS). Several proteins changed in their expression levels and molecular weights. Some proteins were down-regulated or ubiquitinated by parkin over-expression while other proteins were up-regulated by proteasome inhibitor. Characteristics of these parkin-associated proteins will be presented.

**Microscopic aggregations of  $\alpha$ 1A-calcium channel subunit and polyglutamine-containing protein are present mainly in the cytoplasm of the Purkinje cell in SCA6 brain.** *K. Ishikawa<sup>1</sup>, H. Fujigasaki<sup>1</sup>, N. Ohkoshi<sup>2</sup>, T.*

*Makifuchi<sup>3</sup>, T. Arai<sup>4</sup>, K. Hasegawa<sup>5</sup>, T. Kato<sup>6</sup>, S. Shoji<sup>2</sup>, H. Mizusawa<sup>1</sup>.* 1) Dept. Neurology and Neurological Science, Graduate School, Tokyo Medical & Dental Univ, Tokyo, Japan; 2) Dept. of Neurology, Inst. of Clinical Medicine, The University of Tsukuba, Ibaraki, Japan; 3) Dept. of Neuropathology, Saigata National Hospital, Niigata, Japan; 4) Dept. of Applied Biol. Science, Science Univ. of Tokyo, Noda, Chiba, Japan; 5) Dept. of Neurology, National Sagamihara Hospital, Sagamihara, Kanagawa, Japan; 6) 3rd Dept. of Internal Medicine, Yamagata Univ. School of Medicine, Yamagata, Japan.

SCA6 is a dominantly-inherited ataxia caused by an expansion of trinucleotide CAG repeat that code polyglutamine tract in the  $\alpha$ 1A (P/Q-type)-voltage-dependent calcium channel gene. To clarify the site of accumulation of mutant protein and polyglutamine-containing protein in SCA6, we underwent immunohistochemical analysis in 5 SCA6 brains. The  $\alpha$ 1A-calcium channel protein was detected with polyclonal antibody against C-terminal polypeptide of the channel protein, whereas polyglutamine was detected with mouse monoclonal antibody, 1C2. Microscopic aggregation of  $\alpha$ 1A-calcium channel subunit was seen exclusively in the cytoplasm of Purkinje cells, while it was not seen either in the cytoplasm or in the nucleus of any other neurons in SCA6 brains. Numerous 1C2-immunoreactive aggregates were seen in the cytoplasm of SCA6 Purkinje cells, but occasionally smaller 1C2-immunoreactive aggregates were also seen in the nucleus. However, diffuse nuclear staining with 1C2 seen in affected neurons of Machado-Joseph disease (MJD) and dentatorubral-pallidolusian atrophy (DRPLA) were not seen in any Purkinje cells of SCA6 brains. Furthermore, transcription factors TAFII130 or CREB which are positive in nuclear inclusions in MJD, DRPLA and Huntington's disease, were not stained in SCA6 Purkinje cells. The present data suggest that protein aggregation is mainly present, not in the nucleus, but in the cytoplasm of SCA6 Purkinje cells. Therefore, the pathogenic mechanism underlying in SCA6 may be different from that in other polyglutamine diseases.

**Toxicity associated with overexpression of FANCD2 exons 11-13 requires nuclear localization.** *J.A. Hejna, D.A. Bruun, R.E. Moses.* Molec & Med Genetics, L103, Oregon Health Sciences Univ, Portland, OR.

Fanconi Anemia (FA) is a cancer predisposition disorder associated with bone marrow failure, developmental abnormalities and chromosomal instability. Cells from Fanconi anemia patients are hypersensitive to DNA crosslinking agents such as mitomycin C, and also show varying degrees of hypersensitivity to ionizing radiation. Functional complementation of the cellular phenotype by cDNAs has been demonstrated for all of the *FANC* genes cloned to date; however, functional complementation of *FANCD2* cells has only been possible with retroviral constructs because overexpression of *FANCD2* from transfected plasmids is toxic to cells. We have developed an enhanced green fluorescent protein (EGFP) assay to monitor the toxicity of *FANCD2* subclones. Electroporation of CMV-driven *FANCD2*-EGFP fusion constructs into immortalized fibroblast GM639 cells leads to rounding up and cell death within 24 hr. Control electroporations with the pEGFP-N1 vector alone typically achieved greater than 60% transfection efficiency, with uniform fluorescence within 24 hr. Using this assay, we have identified a region of approximately 95 aa, encoded by exons 11-13, that is toxic when transfected into GM639 cells. Furthermore, this region is only toxic when coupled to a nuclear localization signal (NLS), suggesting that the toxicity is mediated by nuclear overexpression of *FANCD2* exons 11-13. The *FANCD2* NLS alone is not toxic in the same assay. Similar results were obtained with a pIRESpuro2 assay, scoring viable colonies after selection on puromycin relative to control transfections. *FANCD2* exon 12 contains a consensus ATM phosphorylation site. We speculate that this site may be required for the toxicity associated with overexpression of *FANCD2*.

**Transcript profiling in Nijmegen breakage syndrome: identification of transcripts induced by ionizing radiation.**

*J.A. Wright, K.M. Cerosaletti, P. Concannon.* Molecular Genetics, Virginia Mason Research Center, Seattle, WA.

Two rare recessive disorders in humans, ataxia-telangiectasia (A-T) and Nijmegen breakage syndrome (NBS) are characterized by a specific sensitivity to the cell-killing effects of ionizing radiation. Milder forms of radiosensitivity are not uncommonly observed among cancer patients undergoing radiotherapy. A rapid test for radiosensitivity could protect these genetically radiosensitive individuals while allowing potentially larger and more efficacious dosages to be delivered to the remaining population. We are exploring the utility of transcript profiling as a tool to identify radiosensitive individuals. As a test system we are using cell lines from NBS patients, and subclones of these lines that have been restored to normal radiosensitivity through the introduction of a functional copy of the NBS1 gene. Microarray hybridization and differential screening of subtracted cDNA libraries from these cell lines have been used to identify transcripts whose expression levels are modulated in response to ionizing radiation exposure. Identified transcripts are then profiled by quantitative PCR over a range of radiation doses and times following radiation exposure. By measuring transcript levels in cells derived from patients with defined DNA damage response defects, such as NBS and A-T, we can categorize transcripts according to the biochemical pathways to which they respond. In NBS cells, we find that genes such as p21 and GADD45 that are regulated normally by p53 are not induced upon irradiation, consistent with a defect in activation of the cellular G1 checkpoint. Similar results were obtained for several S phase inducible transcripts. Unirradiated NBS cells were found to express constitutively high (>10X) levels of several transcripts such as the human homologue of the *Drosophila* smoothed gene that decline to normal levels upon irradiation. Our results define a panel of novel radiation-regulated transcripts that may be of utility in screening patients for radiosensitivity. Their profiling in NBS cells provides new insights into the nature of the DNA damage response defect in this disorder.

**Microarray based expression analysis of mouse cells that lack or overexpress menin.** *N.B. Prasad<sup>1</sup>, E.A. Novotny<sup>1</sup>, J.S. Crabtree<sup>1</sup>, A.G. Elkahoun<sup>1</sup>, S.J. Marx<sup>2</sup>, A.M. Spiegel<sup>2</sup>, F.S. Collins<sup>1</sup>, S.C. Chandrasekharappa<sup>1</sup>.* 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD.

Mutations in *MEN1* lead to multiple endocrine neoplasia type 1 (MEN1), a familial cancer syndrome with endocrine tumors primarily of the parathyroid, anterior pituitary and entero-pancreatic tissues. Other endocrine (foregut carcinoids) and non-endocrine (lipoma, angiofibroma, collagenoma, and ependymoma) tumors are also often associated with MEN1. The 610 amino acid *MEN1*-encoded protein, menin, resides primarily in the nucleus, and is known to interact with the transcription factor JunD. Menin appears to be a typical tumor suppressor, as its inactivation or absence leads to tumors. However, its precise role in tumorigenesis is not yet clear. Here we report our efforts to identify gene expression changes in mouse cells that do not express menin. Since homozygous loss of *Men1* in mice leads to embryonic lethality, we established mouse embryo fibroblast cell lines (MEFs) from 9.5 day embryos with wildtype (wt), heterozygous and null genotypes. The null cells do not express menin as measured by both western and immunofluorescence analysis using menin specific antibodies. RNA isolated from two null and a wt MEF cell lines were used for global expression analysis utilizing mouse cDNA microarrays consisting of 9,000 transcripts. Limiting to a minimum of 3-fold change, we identified 17 transcripts that were up, and 43 that were down, in both the null cell lines compared to wt. We have also established several independent NIH-3T3 mouse fibroblast cell lines that stably overexpress menin up to 8 fold over the endogenous level. RNA from these menin-overexpressing cell lines was also used to generate gene expression profiles using cDNA arrays. Fifteen of the transcripts showing altered expression in menin-overexpressing NIH-3T3 cells were found to be in common with the list of most significant results from the null cells, albeit in the opposite direction, as anticipated. The importance of these transcripts in mapping out the pathway of menin biology can now be explored.



**Transcriptional Analysis of Murine Cardiac Hypertrophy.** *T.D. Gallardo<sup>1</sup>, X. Zhao<sup>1</sup>, G. Esposito<sup>2</sup>, H.A. Rockman<sup>2</sup>, R.V. Shohet<sup>1</sup>.* 1) Internal Medicine, University of Texas Southwestern Medical Ctr, Dallas, TX; 2) Internal Medicine, Duke University Medical Center, Durham, NC.

We have created a cardiac-specific murine cDNA microarray to investigate the transcriptional response during the development of cardiac hypertrophy. We generated a library from adult mouse heart, removed redundancy by a cross-hybridization strategy, and identified the remaining 2500 clones by sequencing. We also removed redundancy from commercially available adult and fetal libraries and spotted all clones, a total of 9100, onto lysine-coated microscope slides using a robotic spotter. RNA was prepared from the left ventricle of mice having similar aortic gradients at 1, 4 and 8 weeks after aortic banding. Pooled samples from 3 mice were compared to hearts from untreated animals. After normalization two main expression trends were recognized. At week one of banding there were 94 cDNAs >3-fold up-regulated and 56 >3-fold down-regulated. By the 4th week only 49 of these up-regulated genes were still regulated to the same degree and by 8 weeks only 30. Interestingly, none of the down-regulated genes were even 1.5-fold down-regulated by week 4. There was a separate group of 16 genes that were not modified at week 1 but were >3-fold up-regulated at 4 weeks. Up-regulation persisted for 7 of these genes at 8 weeks.

We have identified, using a highly representative microarray, two waves of transcriptional regulation that occur after aortic banding. In addition, down-regulation of gene expression appears to rapidly remit during chronic aortic banding despite progressive hypertrophy. Among both sets of regulated genes are novel ESTs. We expect that characterization of these genes and their products will generate new hypotheses about the mechanisms of cardiac hypertrophy as well as targets for analysis of genetic variation in human subjects.

Program Nr: 988 from the 2001 ASHG Annual Meeting

**Identification of eight novel 5-exons in cerebral capillary malformation gene-1 (*CCM1*) encoding KRIT1.** B.A.S. McIntyre, I. Eerola, M. Vikkula. Lab. of Human Molec. Genetics, Christian de Duve Institute, Univ. catholique de Louvain, Brussels, Belgium.

Cerebral capillary malformations (CCM), also known as cerebral cavernomas, are vascular malformations of the brain. These malformations are usually multiple and consist of dilated capillary-venous channels, which tend to grow in size. The average age of clinical presentation is 33 years and the most common symptoms include headache, seizures and cerebral haemorrhage. CCMs often show familial aggregation and genetic linkage has been established to three chromosomal loci, 7q21-22 (CCM1), 7p13-15 (CCM2) and 3q25.2-27 (CCM3).

Identification of mutations in the *CCM1* gene encoding KRIT1 (Krev-1 Interaction Trapped 1) has provided the first clue to the molecular mechanisms causing CCM. In addition, we showed that vascular defects caused by *CCM1* mutations are not restricted to cerebral vasculature, but can also cause cutaneous lesions defined as hyperkeratotic capillary-venous malformations. However, *CCM1* mutations have not been identified in all the families linked to *CCM1*. Here we demonstrate that the *CCM1* gene contains eight additional exons, which may thus encompass missing mutations. (vikkula@bchm.ucl.ac.be).

**A transgenic mouse model for the ANT1 gene.** *V. Tiranti*<sup>1</sup>, *A. Limongelli*<sup>1</sup>, *F. Altruda*<sup>2</sup>, *M. Morbin*<sup>3</sup>, *S. Iussich*<sup>3</sup>, *M. Zeviani*<sup>1</sup>. 1) Divisione di Neurogenetica Molecolare, Istituto Nazionale Neurologico, Milano, Italy; 2) Dipartimento di Genetica, Università di Torino, Torino, Italy; 3) Divisione di Neuropatologia, Istituto Nazionale Neurologico, Milano, Italy.

Heterozygous mutations of ANT1, the heart/muscle isoform of the ADP/ATP translocator, cause autosomal dominant progressive external ophthalmoplegia and multiple mtDNA deletions in the affected tissues. So far, only three ANT1 mutations have been identified, A114P, V289M, and a novel one, L98P. The expression of the A114P substitution in AAC2, the yeast ortholog of ANT1, is associated with reduced or absent growth in glycerol medium. In addition, a KO ANT1<sup>-/-</sup> mouse has been shown to develop combined myopathy and cardiomyopathy with the typical hallmarks of a mitochondrial disease. We have generated a transgenic mouse expressing the A114P mutation in the murine protein, under the control of the heart-specific alpha-myosin heavy chain promoter. The transgenic mice developed an unusual cardiomyopathy, characterized by enormous dilation of the left atrium, hypertrophy of the septum, and profound disorganization of the cardiac syncytium. No defect of the respiratory chain complexes was detected in transgenic hearts. However, a tremendous increase of abnormal mitochondria was observed by both light and electron microscopy. The severity of the cardiac pathology did correlate with the level of expression of the transgene, evaluated by RT-PCR on heart RNA. PCR-based analysis of mtDNA disclosed the presence of multiple rearrangements in transgenic hearts, but not in age-matched control hearts. The mitochondrial cardiomyopathy observed in our model has peculiar features, similar to those found in extremely aged mice. Whether this phenotype is the consequence of the mutation carried by the ANT1 extra-allele, or of an overall increase of ANT1 expression, is currently under investigation.

**Elucidation of the genomic organisation of the bovine Plakoglobin gene and evaluation of its role in a bovine model of arrhythmogenic right ventricular cardiomyopathy (ARVC), associated with woolly haircoat.** *M.A. Simpson<sup>1</sup>, B. Davies<sup>1</sup>, R. Cook<sup>2</sup>, O. Abubaker<sup>1</sup>, M.A. Patton<sup>1</sup>, A.H. Crosby<sup>1</sup>.* 1) Dept Medical Genetics, St Georges Hosp, London, England; 2) NWS Agriculture, Camden, NSW, Australia.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heart muscle disorder characterised by fibrofatty replacement of cardiomyocytes and extracellular matrix with a marked predilection for the right ventricle. The disorder increases the risk of electrical instability leading to ventricular arrhythmias and sudden death. Naxos disease is an autosomal recessive form of ARVC associated with non-epidermolytic palmoplantar keratoderma and woolly hair. We previously mapped the disorder to 17q21 and disease causing mutations were subsequently identified in the plakoglobin gene. The plakoglobin gene encodes a member of the armadillo protein family and is a constituent protein in adherens and desmosomal junctions, with adhesive and signalling functions. An autosomal recessive bovine disorder characterised by ARVC, very similar to that seen in human Naxos disease, has been discovered amongst Australian Poll Herefords. Affected animals also have a woolly haircoat, suggesting that this may represent the first large animal model of Naxos disease and ARVC. In the current study we have identified the bovine homologue of the plakoglobin gene, elucidated its genomic organisation and screened for disease causing mutations in affected cattle.

**Proteomic analysis of vascular smooth muscle cells defines genes involved in the inhibition of apoptosis. V.**

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Apoptosis of vascular smooth muscle cells (VSMC) plays an important role in vascular remodelling, which is one of the major determinants of long-term blood pressure elevation and independent risk factor for cardiovascular morbidity and mortality. Recently we have found that apoptosis in VSMC can be inhibited by inversion of the intracellular Na<sup>+</sup>/K<sup>+</sup> ratio after the sustained blockage of the Na-K-ATPase. Using the proteomic approach we have performed a comparative analysis of protein expression patterns of control VSMC and of those with Na-K-ATPase inhibited by ouabain. Soluble, membrane bound, and cytoskeleton proteins of VSMC separated by the detergent extraction, were resolved by two-dimensional electrophoresis. We found that ouabain treatment led to overexpression of nearly 100 soluble and 15 membrane-bound proteins. Among proteins, which showed the highest level of expression we identified several members of the heat shock protein superfamily HSP70 which suggests that these proteins may participate in a direct or indirect inhibition of apoptosis. This hypothesis was confirmed by the transient expression of cDNA coding for HSP70 protein, which caused inhibition of apoptosis in VSMC triggered by their deprivation from growth factors.

**Differences of intracellular localization and molecular size indicate that posttranslational modification of overexpressed BIPs is different from native BIPs.** *W. Ju<sup>1</sup>, R. Kascsak<sup>2</sup>, W.T. Brown<sup>1</sup>, N. Zhong<sup>1,3</sup>.* 1) Dept. Human Genetics, New York State Inst Basic Res, Staten Island, NY; 2) Laboratory of Monoclonal Core2, NYS Inst Basic Res, Staten Island, NY; 3) Dept. Neurology, SUNY-Health Science Center at Brooklyn, Brooklyn, NY.

BIPs, a protein with one transmembrane domain, which we previously identified and cloned, shows a slow interaction with CLN3-encoded protein Battenin. Battenin is deficient in Batten disease, the juvenile form of neuronal ceroid lipofuscinoses (JNCL) and characterized by lysosomal lipofuscin storage. Overexpressing BIPs by fusing BIPs N-termini with 6 x Histidines in plasmid pcDNA 6 transformed into human neuron precursor cells NT2, and into Cos-7 cells showed that BIPs could be detected as a cellular membrane protein with two forms, ~52-kDa and ~60-kDa, by anti-His antibody. However, using antibodies generated against two peptides selected from the N-terminal region of BIPs, we found that both protein localization and molecular size for native BIPs are quite different from the overexpressed and fused BIPs. Immunostaining signals for native BIPs were cytoplasmic, as well as peri-nuclear, in a punctate vesicular fashion. Two protein bands, with molecular sizes of ~89-kDa and ~105-kDa, were detected on western blots. Our results are similar to the immunochemical studies of Battenin, for which the native protein is localized at mitochondria but the overexpressed protein was found in lysosome, ER, and Golgi bodies. These results indicate that the overexpressed protein has a different posttranslational modification than the native protein, which results in protein mislocalization.

**Identification of genes differentially expressed in the fragile X syndrome.** *I. Gantois, E. Reyniers, R.F. Kooy.*

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The fragile X syndrome is caused by the absence of the FMR1 protein, FMRP. FMRP is expressed in most cell types but shows highest expression in neurons and Sertoli cells. In the cell FMRP may shuttle between nucleus and cytoplasm, perhaps transporting a specific subset of brain mRNAs to the actively translating ribosomes near the synaps in neurons. To study the function of the protein, a fragile X knockout mouse was created several years ago. This mouse model shows some characteristics compatible with the symptoms of human patients, including macroorchidism, spatial learning deficit and dendritic spine abnormalities.

To increase our understanding how absence of FMRP leads to mental retardation, sequences differentially expressed between fragile X knockout mouse and control mouse were isolated using the differential display technique. This method allows comparison of the expression levels of >95% of all neuronal genes between the two genotypes. Using differential display, we isolated 143 sequences underexpressed in knockout and 81 overexpressed in knockout with a length between 200-1100 bp. Differential expression of some of the sequences was verified using micro arrays. These partial cDNAs were sequenced, and we searched for homologies of these sequences in EST and gene databases. For some sequences, homologies with known genes in the database were found.

A study of the function of these differentially expressed genes may help us understand how absence of FMRP relates to the clinical symptoms of the fragile X syndrome.

**Hirschsprung disease, microcephaly, mental retardation, and dysmorphic facial features caused by loss-of-function mutations in SIP1 (Smad Interacting Protein 1).** V. CACHEUX<sup>1</sup>, F. DASTOT-LE MOAL<sup>1</sup>, H.

KAARIAINEN<sup>2</sup>, N. BONDURAND<sup>1</sup>, R. RINTALA<sup>3</sup>, B. BOISSIER<sup>1</sup>, O. CAMAND<sup>4</sup>, G. BASSEZ<sup>1</sup>, M. ABITBOL<sup>4</sup>, M. WILSON<sup>5</sup>, D. MOWAT<sup>6</sup>, M. GOOSSENS<sup>1</sup>. 1) INSERM U468 et AP-HP, Hopital Henri Mondor, Creteil, France; 2) Department of Medical Genetics, The Family Federation of Finland, Helsinki, Finland; 3) Helsinki University Children's Hospital, Helsinki, Finland; 4) CERTO, Faculte de Medecine de Necker, 75015 Paris, France; 5) Department of Clinical Genetics, The Children's Hospital at Westmead, Sydney, Australia; 6) Department of Medical Genetics, Sydney Children's Hospital, Randwick, and School of Paediatrics, University of New South Wales, Sydney, Australia.

Hirschsprung disease (HD), or aganglionic megacolon is a relatively common disorder resulting from the failure of normal neural crest cells development. The genetic aetiology of this neurocristopathy is complex all the more because HD is associated with a variety of other congenital disorders. In particular, HD has been described in association with microcephaly, mental retardation and characteristic facial features, delineating a syndromic form of HD. Taking advantage of a de novo translocation breakpoint at 2q22, we identified SIP1 (Smad interacting protein 1), a two-handed zinc finger/homeodomain transcription factor, as the gene disrupted by the chromosomal rearrangement. Here, we report on 6 unrelated patients with the same phenotype, which bear heterozygous frameshift mutations of SIP1 gene causing early truncation of the protein. Most of the SIP1 mutations described in this syndrome lie in exon 8, delineating a hot spot region for mutations. Preliminary expression studies show that SIP1 mRNA is detected in almost all adult human tissues tested (heart, brain, placenta, lung, liver). To help unravel its physiological role during development, we studied SIP1 gene expression in embryonic and fetal mouse and human tissues by in situ hybridization. These experiments allowed to precisely map SIP1 early expression in fetal brain, a finding relevant to the phenotypic features that constitute this syndrome.



**Satellite 2 methylation patterns in normal and ICF syndrome cells and association of hypomethylation with advanced replication.** *K.M.A. Hassan*<sup>1</sup>, *T. Norwood*<sup>2</sup>, *G. Gimelli*<sup>3</sup>, *S.M. Gartler*<sup>1</sup>, *R.S. Hansen*<sup>1</sup>. 1) Medicine, University of Washington, Seattle, WA; 2) Pathology, University of Washington, Seattle, WA; 3) Laboratorio de Citogenetica, Istituto G. Gaslini, Genova, Italy.

Mutation in the *DNMT3B* DNA methyltransferase gene is a common cause of the ICF immunodeficiency syndrome and leads to hypomethylation of satellites 2 and 3 in pericentric heterochromatin. This hypomethylation is associated with centromeric decondensation and chromosomal rearrangements, suggesting that these satellite repeats have an important structural role. In addition, the satellite regions may have functional roles in modifying gene expression. The extent of satellite hypomethylation in ICF cells is unknown because methylation status has only been determined with restriction enzymes that cut infrequently at these loci. We, therefore, developed a bisulfite conversion-based method to determine the detailed cytosine methylation patterns at satellite 2 sequences in a quantitative manner for normal and ICF samples. From our sequence analysis of unmodified DNA, the internal repeat region analyzed for methylation contains an average of 17 CpG sites. The average level of methylation in normal lymphoblasts and fibroblasts is 69% compared to 20% in such cells from ICF patients with *DNMT3B* mutations, and 29% in normal sperm. Although the mean satellite 2 methylation values for these groups do not overlap, there is considerable overlap at the level of individual DNA strands. Our analysis also revealed a pattern of methylation specificity, suggesting that some CpGs in the repeat are more prone to methylation than other sites. Variation in satellite 2 methylation among lymphoblasts from different ICF patients prompted us to determine the frequency of cytogenetic abnormalities in these cells. Although our data suggest that some degree of hypomethylation is necessary for pericentromeric decondensation, factors other than DNA methylation appear to play a major role in this phenomenon. Another such factor may be altered replication timing because we discovered that the hypomethylation of satellite 2 in ICF cultures is associated with advanced replication time.

**A necdin-like gene (*NDNL2*) in the chromosome 15 autism susceptibility region: expression, imprinting, and mapping of the human and mouse orthologues.** R. Wevrick, J.M. Bischof, T.K. Chibuk. Dept Medical Genetics, Univ Alberta, Edmonton, AB., Canada.

Human chromosome 15q is prone to cytogenetic rearrangements, in part due to repetitive elements located therein. Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are two neurodevelopmental disorders caused by deletions of 15q11-q13, and neurodevelopmental abnormalities are associated with supernumerary chromosomes derived from inverted duplications of 15q. In addition, an autistic disorder susceptibility locus has been localized to proximal 15q by linkage and association studies. While AS is strongly associated with *UBE3A* mutations, multiple candidate genes for PWS have emerged and are likely to have an additive effect in causing this disorder. In particular, two members of the *NDN/MAGE* gene family, *NDN* and *MAGEL2*, are located in the PWS deletion region and are not expressed in individuals with PWS. *NDN* and *MAGEL2* may be implicated in the PWS phenotype as they are expressed in the developing nervous system. We have therefore investigated the possibility that other *NDN/MAGE* genes may also be present on proximal 15q and may be involved in neurodevelopmental disorders. Indeed, a novel necdin-related gene, *NDNL2*, is found on proximal 15q within the critical region for autistic disorder susceptibility. We have examined the expression of *NDNL2* and its murine orthologue, and placed the murine gene on mouse chromosome 7 in the region of conserved synteny with other PWS region genes. The proximal region of chromosome 15 is subject to genomic imprinting, the expression of a gene from only one allele depending on parent-of-origin. Furthermore, the maternal derivation of chromosomal abnormalities seen in individuals with autistic disorder suggests a parent-of origin effect. We have also analyzed the imprinting of human and mouse *NDNL2/Ndnl2*. In summary, we have identified a candidate gene for the neurodevelopmental abnormalities associated with proximal human chromosome 15.

**3-Hydroxyisobutyrate dehydrogenase gene: characterization of the cDNA, structural organization, mapping of the locus to 7p15, and mutation analysis.** *K. Yuji<sup>1</sup>, S. Ogawa<sup>1</sup>, A. Hangaishi<sup>1</sup>, H. Ueno<sup>1</sup>, M. Gibson<sup>2</sup>, W. Nyhan<sup>3</sup>, S. Yamaguchi<sup>4</sup>, M. Kimura<sup>4</sup>, M. Sasaki<sup>5</sup>, S. Ikawa<sup>6</sup>, H. Hirai<sup>1</sup>.* 1) Dept. of Hematology, Graduate School of Medicine, Univ. of Tokyo, Tokyo, Japan; 2) Biochemical Genetics Laboratory, Oregon Health Sciences Univ., Portland, OR; 3) Division of Biochemical Genetics, UCSD School of Medicine, La Jolla, CA; 4) Dept. of Pediatrics, Shimane Medical University, Shimane, Japan; 5) Dept. of Child Neurology, National Center of Neurology and Psychiatry, Tokyo, Japan; 6) Dept. of Cell Biology, IDAC, Tohoku Univ., Miyagi, Japan.

3-Hydroxyisobutyrate dehydrogenase (3HIBADH, 3-hydroxy-2-methylpropanoate: NAD<sup>+</sup> oxidoreductase, EC 1.1.1.31) catalyzes an NAD<sup>+</sup>-dependent, reversible oxidation of 3-hydroxyisobutyrate to methylmalonate semialdehyde in the pathway of L-valine. To date, ten cases of human 3HIBADH deficiency (3-hydroxyisobutyric aciduria, 3HiB uria, [MIM 236795]) have been reported. By screening testis cDNA library, we isolated and characterized a 1.9kb human HIBADH cDNA with a 1008bp (336-amino acid) open reading frame showing 91% amino acid identity to rat 3HIBADH. Determination of the gene structure reveals that the human 3HIBADH gene spans about 137kb with 8 exons and 7 introns, and maps to chromosome 7p15. Northern blot analysis showed a ubiquitous expression of 3HIBADH in all adult tissues. Four patients with 3HiB uria were analyzed for sequence variations, but we could not find any mutations in the human 3HIBADH gene. Functional investigation of the 3HIBADH gene and its potential involvement in 3HIBADH deficiency are in progress.

**Spidey: An mRNA-to-genomic alignment program.** *S.J. Wheelan<sup>1,2</sup>, D.M. Church<sup>1</sup>, J.M. Ostell<sup>1</sup>.* 1) National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894; 2) Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Expressed sequences are an incredible resource, but even more information can be gained by locating their genomic sources. By aligning an expressed sequence to its parent genomic sequence, a researcher can locate a genes position, can identify potential regulatory elements, and can study paralogs or pseudogenes. These alignments are not trivial; sequence polymorphisms and repeats (especially at the ends of exons) make the problem more difficult, even after the correct genomic sequence has been found amid all the distracting sequences.

Cross-species spliced alignments are also quite useful, especially from a medical or developmental perspective. Many tissues representing many developmental stages are available from experimental organisms and not from humans, and these tissues may harbor uniquely informative expressed sequences. Evolutionary divergence increases the difficulty of achieving correct and useful cross-species spliced alignments.

We have developed a spliced alignment program, Spidey, that can quickly produce reliable mRNA-to-genomic alignments. Spidey can also compute cross-species comparisons.

We first tested Spidey's accuracy and ability to ignore nearby related sequences by aligning mRNAs from gene clusters back to the genomic sequence. We then took mRNAs derived from annotations and aligned them back to their genomic sequences. We also wanted to test Spidey's ability to perform interspecies alignments accurately; to this end, we aligned mouse orthologs to human genomic sequence. In an effort to test Spidey's efficacy using real data, we took a set of 11,000 sequences from LocusLink and aligned them to the NCBI human genome assembly based on the December 7 data freeze.

Spidey is available as a web service and an executable at <http://www.ncbi.nlm.nih.gov/spidey>.

**Analysis of differential gene expression during development of cardiac hypertrophy.** *T. Hahn, I. Hansmann, M. Schlicker.* Institut für Humangenetik, Martin-Luther-Universität, Halle, Germany.

The spontaneously hypertensive rat (SHR) is a well-known model for the study of hypertension and heart failure. Diverse quantitative trait loci (QTLs) for hypertension and cardiac hypertrophy indicate that different chromosomal areas account for the development of the two diseases. While the development of the clinical symptoms the SHR passes through distinct phenotypic stages. Up to four weeks after birth the animals are normotensive, and then males firstly evolve hypertension (12 weeks) and afteron hypertrophy of the left heart ventricle (26 weeks). To identify candidate genes that contribute to the initiation or the progression of cardiac hypertrophy we screened the hearts of these SHRs for differentially expressed genes. To use a high-resolution method we established a subtractive hybridization system based on cDNA selection and suppression PCR. The subtractive hybridizations from the 4- to the 12- and to the 26-weeks stages resulted in 145 different cDNA clones. The following differential screening suggested 56 cDNAs to be candidates for differentially expressed genes. Northern blot analyses of meanwhile 42 cDNAs identified 16 genes which are upregulated at their expression level in comparison to the wild type Wistar-Kyoto rat. According to homology searches in electronic databases we identified so far 8 novel genes that are differentially expressed in hypertrophic growing SHR hearts. The parallel identification of known genes which are discussed to be involved in cardiomyopathy (g-sarcoglycan) or cardiac hypertrophy (acyl coenzyme A dehydrogenase) points to the efficiacy of the chosen method. Next, we will check the chromosomal localization of these genes in respect to known QTLs for cardiac hypertrophy by FISH analysis and analyze the expression and integrity of the novel identified genes in animals with cardiac hypertrophy.

**Identification of a novel mammalian DM-domain containing gene, candidate for premature ovarian failure.** C. Ottolenghi<sup>1</sup>, R. Brauner<sup>2</sup>, E. Thibaud<sup>2</sup>, K. McElreavey<sup>1</sup>. 1) Immunogenetique Humaine, Institut Pasteur, Paris, France; 2) Pediatric Endocrinology, Necker Hospital, Paris, France.

Premature ovarian failure (POF), defined as a condition causing amenorrhea, hypoestrogenism and elevated gonadotropins before the age of 40, is estimated to have an incidence of 2-3%. In approximately 60% of all POF patients the underlying cause cannot be identified and these cases are classified as idiopathic. Recent epidemiological studies suggest that up to 30% of cases may be familial. POF, primary amenorrhoea and ovarian dysgenesis can occur within the same family indicating that although they represent a spectrum of phenotypes, a common genetic cause is suspected. Deletions of the long arm of the X chromosome are associated with both primary amenorrhea and POF. Three regions have been defined associated with ovarian failure, i.e. Xq13-21 (POF2), Xq22-25 (POF1) and Xq26.2-28 (POF3). The *Drosophila* doublesex (*dsx*) and *Caenorhabditis elegans* *mab-3* gene products perform several related regulatory sex-specific functions, which are at least partially interchangeable in vivo. A unique DNA-binding domain (the DM domain) is shared by Doublesex and Mab-3. Vertebrate DM genes have likewise been identified and, remarkably, one of them is associated with anomalies of early testis differentiation. In *Drosophila* sexual differentiation is controlled by alternative splicing of doublesex mRNA resulting in male and female specific isoforms which direct sexual differentiation. We describe the characterisation of a novel mammalian DM-domain containing gene, DMRTC1, which may be structurally and functionally related to the female-specific isoform of doublesex. This gene is expressed in the human ovary and localised in Xq13. We propose that DMRTC1 is a positional and evolutionary candidate for the POF2 condition.

**Human GH Transcripts are Alternatively Spliced in Different Tissues of Transgenic Mice.** *C.T. Moseley<sup>1</sup>, M.A. Prince<sup>1</sup>, L.K. Hedges<sup>1</sup>, R.C. Ryther<sup>1</sup>, J.G. Patton<sup>1</sup>, W.G. Beamer<sup>2</sup>, J.A. Phillips III<sup>1</sup>.* 1) Dept Pediatrics, Division of Medical Genetics, Vanderbilt Univ, Nashville, TN; 2) The Jackson Laboratory, Bar Harbor, ME.

Human growth hormone (GH) is produced by pituitary somatotroph cells. GH enhances tissue proliferation and maturation and exerts a variety of metabolic effects. GH mRNA is alternatively spliced to yield different isoforms that encode 22, 20, 17.5 and 11.3 kDa translation products. Mature (22 kDa) GH is translated from the mRNA isoform that includes exons 1-5. The 20 and 17.5 kDa isoforms have deletions of the first 15 or all codons of exon 3, respectively, and the 11.3 kDa isoform has exons 3 and 4 deleted. We have discovered a series of GH gene mutations that cause GH deficiency because they perturb intron (ISE) or exon (ESE) splicing enhancers and derange alternative splicing. We hypothesize that alternative splicing of GH transcripts is regulated in a tissue-specific manner. We determined the patterns of GH alternative splicing by quantitative RT-PCR analysis of human GH transcripts in different tissues from transgenic mice. RNA was isolated from frozen tissues, and human GH specific primers in exons 1 and 5 were used to PCR amplify cDNAs. The amounts of isoforms were quantitated by comparison of peak locations and areas on an ABI 310 using a FAM labeled primer. Relative amounts of 22, 20, 17.5 and 11.3 kDa transcripts were determined to be 83, 11, 5 and 1%; 88, 10, trace amounts (TA) and 1%; and 87, 12%, TA, and TA; in pituitary, liver and kidney, respectively. Our data indicate that: 1) consistent, tissue specific patterns of alternative splicing of human GH transcripts occurs in a transgenic mouse model and 2) the resulting transcripts could result in different GH proteome patterns for each tissue. Our results suggest that: 1) the tissue specific patterns of alternative splicing may result from tissue specific variations in the amounts of serine/arginine (SR) rich or other proteins or factors that promote alternative splicing, and 2) many of the estimated 22-34% of all human genes that are alternatively spliced may have tissue specific regulation.

**Heterogeneously reduced expression of calcium receptor, vitamin D receptor and parathyroid hormone mRNAs in parathyroid glands of secondary hyperparathyroidism.** *S.V. Valimaki<sup>1</sup>, F. Farnebo<sup>1</sup>, L. Forsberg<sup>1</sup>, C. Larsson<sup>1</sup>, L.O. Farnebo<sup>2</sup>.* 1) Molecular Medicine, Endocrine Tumor Unit, Stockholm, Sweden; 2) Department of Surgery, Karolinska Hospital, stockholm, Sweden.

Secondary hyperparathyroidism (2HPT) arises in patients with chronic renal failure and is characterized by inappropriate control of parathyroid hormone (PTH) secretion and asymmetric hyperplasia of the parathyroid glands. Receptors for calcium and vitamin D are involved in the control of secretion as well as parathyroid cell proliferation. Defective receptor mechanisms may therefore play a role in the pathogenesis of 2HPT. Previous reports have shown decreased expression levels of expression of calcium receptor (CaR), calcium sensing receptor (CAS) and vitamin D receptor (VDR) protein and mRNA in hyperplastic parathyroid glands of 2HPT when compared with normal parathyroid glands whereas the expression pattern between and within different glands remains unknown. **Methods.** In order to investigate the expression of CaR, CAS, VDR and PTH mRNAs we analyzed 36 hyperplastic glands from 18 patients with 2HPT with *in situ* hybridization. In nine nodular parathyroid glands, it was possible to make a comparison between the expression of these mRNAs in nodular and internodular areas. **Results.** The level of CaR was similar in the hyperplastic glands as in the biopsies of normal parathyroid, while the levels of CAS, VDR and PTH were clearly reduced in the hyperplastic glands. There was a positive correlation between the expression of CaR and CAS ( $p=0.02$ ). Otherwise, no correlations between CaR, CAS, VDR and PTH mRNAs were found. The expression of all four genes was highly variable as well between different glands as within individual glands. **Conclusion.** This study demonstrates a heterogeneously decreased expression of four receptor mRNAs that are of importance in the control of PTH secretion and parathyroid cell proliferation in parathyroid glands of 2HPT. The expression pattern corroborates earlier studies in which it has been assumed that each nodule in secondary HPT is of monoclonal origin, but that the monoclonal origin of each nodule is independent.



**AF-1 specific coactivators of steroid hormone receptors.** *L.B. Tovar y Romo, E. Langley, A. Leon Del Rio.*  
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Steroid hormone receptors are ligand dependent transcriptional regulators capable of controlling gene expression in many biological processes. Transcriptional activity is mediated through two transcriptional activation functions, AF-1 and AF-2, in the amino and carboxyl regions of the receptor, respectively. These transcriptional activation functions are modulated by transcriptional coregulators which can enhance or repress transactivation. However, very few have been reported to interact specifically to the AF-1 region of nuclear receptors. We utilized the yeast two hybrid system to identify proteins which interact with the AF-1 of the estrogen receptor (ER). We have begun the characterization of two proteins which interact with this region: SRY-interacting protein (SIP-1) and Myc associated zinc finger protein (MAZ). Transcriptional activation studies show that SIP-1 functions as a coactivator, not only for the ER, in a number of cell types, but for the androgen and progesterone receptors as well. SIP-1 increases transactivation by the estrogen receptor by up to 200% in MCF-7 cells. In CV-1 cells, SIP-1 increases transactivation by the androgen and progesterone receptors by up to 600%. In contrast, MAZ seems to act as a corepressor for these receptors. The AF-1 region of ER is involved not only in the estrogenic response of target genes, but is also required for inhibition of ER activity by tamoxifen (TOT). TOT is the most common treatment for patients with ER-positive breast cancer. However, although treatment with TOT is highly effective, eventually there is an acquired resistance to this compound and, in many cases, TOT can now act as an ER agonist. Identification of proteins involved in AF-1 activation could lead to a further understanding of the mechanisms by which TOT can have differential activities in different tissues and in different stages of breast cancer.

**Dose-dependent Shift from Repressor to Activator in a DAX1 Variant from a Female with Adrenal Hypoplasia Congenita.** *J.J. Wilson<sup>1</sup>, B.D. Bowling<sup>1</sup>, J. Phelan<sup>2</sup>, Y.H. Zhang<sup>2</sup>, B.L. Huang<sup>2</sup>, E.R.B. McCabe<sup>2</sup>, E. Vilain<sup>1, 2</sup>.* 1) Human Genetics, UCLA, Los Angeles, CA; 2) Pediatrics, UCLA, Los Angeles, CA.

Mutations in the nuclear receptor DAX1 are responsible for both X-linked adrenal hypoplasia congenita (AHC) and male-to-female XY sex reversal. All missense mutations discovered to date alter DAX1 repression of SF1 activity, another orphan nuclear receptor, and have been limited to the C-terminal hydrophobic core of the putative ligand binding domain (LBD). We identified a family in which an unaffected father and his daughter with AHC both harbor a DAX1 sequence variation: cysteine-200 changed to tryptophan (C200W). The sequence variant was not found in over 100 X chromosomes analyzed within an ethnically mixed population. C200W is the first missense mutation to be localized upstream of the classic nuclear receptor LBD, within a region that harbors novel sequence repeats that display RNA-binding capability *in vitro*. Three sequential consensus SF1 response elements were utilized upstream of a luciferase reporter construct in order to demonstrate the classic DAX1-mediated silencing of SF1 trans-activation. Upon co-transfection of equimolar concentrations of the C200W DAX1 mutant with SF1 in embryonic kidney cell culture (293T), a complete wild-type repression of reporter expression was found. However, as the C200W concentration was incrementally increased, a concomitant loss of silencing function became apparent. High concentrations of C200W enhanced luciferase expression to levels exceeding those seen with SF1 activation alone. In summary, the unique C200W variant, associated with a normal and an AHC phenotype, converts DAX1 activity from antagonist to agonist. Since the father carries the variant and does not exhibit AHC, this mutant may be acting through variable co-factor complexes represented within the different genetic backgrounds. Our studies on this mutant suggest a repressor role for DAX1 in a complex and robust network critical for the developing reproductive axis. This work supports the emerging theme in biology that normal development is shaped by powerful inhibitory forces and molded by transient surges of relief from this silencing repression.

**Functional delineation of the COOH-terminal tail of the human cystic fibrosis transmembrane conductance regulator (CFTR) using a combined analysis.** *C. Ferec, J.M. Chen.* INSERM-EMI 01 15, Etablissement Français du Sang-Bretagne, Université de Bretagne Occidentale, and Centre Hospitalier Universitaire, 46 rue Félix Le Dantec, 29275 Brest, France.

Since the cystic fibrosis transmembrane conductance regulator (CFTR) gene was cloned in 1989 (Riordan et al.), an enormous wealth of knowledge about its encoded 1480 amino acid protein, including evolutionary, mutational, functional, and structural data, has been accumulated. These data, evaluated together, have recently enabled us to define a "functional R domain" (Chen, Scotet, and Ferec 2000). Importantly, this new definition not only agrees well with that derived from a concurrent functional approach (Csanady et al. 2000), but also appears to concur with the regulatory role of this unique domain (Baldursson, Berger, and Welsh 2000). Here, we extend to refine the COOH-terminal tail (C-tail) of the CFTR protein by exploiting the same approach. Taking into accounts the current available evolutionary data, clinical observations and functional studies, we could confidently assign residue E1418 as the NH<sub>2</sub> boundary of the C-tail, which is 31 amino acid downstream the original definition (T1387). This new definition can best delineate the C-tail's functional role in the CFTR. On the one hand, the newly defined C-tail is "non-essential", as the effects on the biogenesis, processing and stability of the CFTR previously assigned to the original C-tail have now been re-located to the preceding sequence. On the other hand, the newly defined C-tail does appear to be "essential", as the well conserved motif involving the final three amino acids (T1478-R1479-L1480) represents a PDZ-interacting domain which is required for the polarization of CFTR to the apical plasma membrane in epithelial cells. Nevertheless, the new defined C-tail as a whole would play a minor role in the pathogenesis of CF disease. Indeed, neither the S1455X nor the Q1476X caused a severe CF phenotype; and none of the three missense mutations (R1422W, D1445N and R1453W) identified in this region can be confidently assigned as causal for CF disease. The usefulness of this basic approach may be further exploited in the NBD1 and NBD2 domains.

**Beta-defensin 1 gene variability among non-human Primates.** *S. CROVELLA*<sup>1</sup>, *M. BONIOTTO*<sup>1</sup>, *M. DEL PERO*<sup>2</sup>, *A. AMOROSO*<sup>1</sup>. 1) Molecular Genetics Unit. Department of Sciences of Biology and Reproduction . University of Trieste. Italy; 2) Department of Animal Biology, University of Turin. Italy.

Defensins are a family of peptides that play an important role in innate immunity. Defensins exhibit a broad spectrum of antimicrobial activities against bacteria and fungi. Two families have been identified so far in mammals, a-defensins and b-defensins, presumably derived from a common ancestral defensin. A long term study on the evolution of these multigene families among primates has been undertaken to investigate i) the degree of interspecific differentiation; ii) the genetic mechanisms responsible for the variability of these molecules; and iii) the possible role of different environmental factors in their evolution. In this study we sequenced the coding regions of the b-defensin 1 gene in 16 primate species. Nucleotide sequences have been obtained from apes, several African and Asian catarrhine monkeys and one New World monkey. A comparison of rates of synonymous and nonsynonymous nucleotide substitution indicates that the primate b-defensin 1 gene evolved under a pattern of random nucleotide substitution as predicted by the neutral theory of molecular evolution. These results are not consistent with the hypothesis that defensins have diversified in response to changes in the microbial species to which a given host is exposed. Analyses of the gene's interspecific variability have yielded some insights about the pattern of molecular evolution of the gene among primates. Humans and apes present high levels of sequence similarity, differing in only one amino acid residue in the mature peptide. Compared with these taxa, hylobatids and cercopithecids exhibit 3-4 amino acid substitutions, which increase the net charge of the active molecule.

**Molecular Evolution of MC1R Gene in human and non-human Primates.** *X-M. Lu, Y-P. Zhang.* Lab. Cellular & Molec. Evol., Kunming Inst. Zool., Chinese Academy of Sciences, Kunming, Yunnan 650223, People's Republic of China.

It is widely assumed that genes that influence variation in skin and hair pigmentation are under selection. The melanocortin receptor 1 (MC1R) plays a central role in regulation of eumelanin (brown/black melanins) and pheomelanin (red/yellow melanins) synthesis in the mammalian melanocyte. Many substitutions have been observed in human MC1R gene, a few of which may associate with variation of skin and hair color. To understand the sequence variation pattern of MC1R gene in human population and to study the evolution of this gene, we sequenced the MC1R gene from 25 nonhuman primates: 4 in Hominoidea, 17 in Cercopithecidae (Old world monkey), 3 in Cebodae (New world monkey) and one in Loridea. Based on these nucleotide and amino acid sequences and the published sequences from humans and other primates, the phylogenetic trees were constructed using parsimony and maximum likelihood methods. The evolutionary rate was estimated and then neutral selection hypothesis was tested. Our results indicated that: (1) nearly neutral selection was showed in Hominoid and the Old World monkeys, but not in the New World monkeys; and (2) the evolutionary rate in the Hominoid is significantly higher than that in the Old World monkeys, which conflicted with the Hominoid slowdown hypothesis.

**SCA7 locus information drives trinucleotide repeat instability in transgenic mice.** *R.T. Libby<sup>1</sup>, R. Lau<sup>2</sup>, D.D. Einum<sup>3</sup>, K. Nichol<sup>2</sup>, L.J. Ptacek<sup>3,4</sup>, Y.H. Fu<sup>5</sup>, C.E. Pearson<sup>2</sup>, A.R. La Spada<sup>1</sup>.* 1) Dept Lab Medicine, Univ Washington, Seattle, WA; 2) Dept Genet, Hosp for Sick Children, Toronto, Ont, CA; 3) Dept Human Genet, Univ Utah, Salt Lake City, UT; 4) HHMI, Univ Utah, Salt Lake City, UT; 5) Dept Neurobiol & Anat, Univ Utah, Salt Lake City, UT.

Spinocerebellar ataxia type 7 (SCA7) is caused by a trinucleotide repeat expansion, a class of mutations characterized by intergenerational and somatic genetic instability. Of the CAG repeat disorders, SCA7 shows the most extreme degree of anticipation. To understand the molecular basis of CAG repeat instability, we chose to model the ataxin-7 repeat in mice by generating two types of transgenic constructs. The first construct (RL-) consisted of a 13.5 kb Sal I-Spe I genomic fragment from the human ataxin-7 gene, while the second construct placed the coding region of the human ataxin-7 cDNA into an expression cassette (PrP-). In both cases, an expanded SCA7 allele with 92 CAG repeats was used. We compared intergenerational and somatic instability in 5 independent lines of mice (2 RL-SCA7-92Q lines & 3 PrP-SCA7-c92Q lines) by capillary and polyacrylamide gel electrophoresis of PCR-amplified repeats. Although we observed marked intergenerational instability in RL-SCA7-92Q mice with 87% of parent-offspring transmissions showing alterations, the PrP-SCA7-c92Q mice showed limited instability with changes in just 14% of transmissions ( $p < .001$ ). While the length shifts were modest in the PrP-SCA7-c92Q mice {mean length change = -0.2; range = -4 to +5} and there was a slight tendency toward contractions {expansion:contraction = 2:3}, the RL-SCA7-92Q mice showed a marked expansion tendency with 90% of size shifts being expansions {mean length change = +2.8; range = -4 to +11}. The differences in instability between the two constructs were also observed somatically, with the RL-SCA7-92Q lines showing more instability in testes and liver. Based on these results, we conclude that SCA7 genomic context explains the significantly greater repeat instability. Furthermore, there must be cis-acting information within the genomic fragment used to generate the RL-SCA7-92Q mice that is responsible for this difference in repeat instability.

**Fiber-FISH analysis of gene organization and rearrangements at the Rhesus blood group locus.** *Y. Suto*<sup>1</sup>, *Y. Ishikawa*<sup>2</sup>, *H. Hyodo*<sup>2</sup>, *M. Uchikawa*<sup>2</sup>, *M. Hirai*<sup>3</sup>, *T. Juji*<sup>2</sup>. 1) Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo; 2) Department of Research, the Japanese Red Cross Central Blood Center, Tokyo; 3) Department of Integrated Biosciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo.

Multicolor fluorescence *in situ* hybridization on released chromatin DNA fibers (fiber-FISH) is a powerful tool for visual examination of the overall gene structure and arrangement at a single cell level. Using two DNA probes corresponding to introns 3 and 7 of a human *RH* gene in the analysis of the *RH* locus in healthy humans, we revealed the first evidence that the Rhesus (Rh) blood group locus of typical Rh-positive individuals is composed of two *RH* genes in a tail-to-tail arrangement [telomere - *RHCE* (5'→3') - *RHD* (3'→5') - centromere] within a region of less than 200 kb on chromosome 1p36.1, while typical Rh-negative individuals have a single *RHCE* gene (Suto *et al.*, 2000). When these probes were used in the analysis of the *RH* locus in five chimpanzees, it was found to be homozygous for either two or four *RH* genes. In this study, we further examined the *RH* locus of nine healthy Japanese donors who were serologically Rh-negative but *RHD*-positive by polymerase chain reaction with sequence-specific priming (PCR-SSP assay) on exons 7 and 10, and intron 4. They were shown to have different genomic organizations including partial deletion, duplication, recombination and mosaicism within the *RH* locus, which were likely to be responsible for RhD-negative expression but not necessarily dependent on their serological phenotype. As the frequency of each serological phenotype varies among different races or populations, a subsequent study on other Asian and African populations is underway.

**Towards understanding the functional role of the laforin protein in Progressive Myoclonus Epilepsy.** *L. Ianzano, X.C. Zhao, E. Chan, E.J. Young, D.M. Andrade, B.A. Minassian, S.W. Scherer.* Dept Genetics, Hosp Sick Children, Toronto, ON, Canada.

Lafora disease is an autosomal recessive form of adolescent progressive myoclonus epilepsy (PME) characterized by myoclonias, decreasing mental function, and death within 10 years of onset. The diagnosis of Lafora disease is based on detection of periodic acid Schiff-positive polyglucosan inclusions (Lafora bodies), which are present in various tissues including brain, suggesting LD might be a generalized storage disease. The EPM2A gene at chromosome 6q24 has been shown to be involved in Lafora disease and it encodes a novel dual-specificity phosphatase (DSP) named laforin. Immunofluorescence and electron microscopic studies on laforin expressed in Cos cells revealed its association with the rough endoplasmic reticulum. To gain insight into the possible role of laforin in the pathology of the disease we are working to identify interacting proteins using yeast two-hybrid screens, immunoprecipitation and GST pull-down experiments. The full-length EPM2A cloned into a pGBKT7 vector was used as a bait to screen a human brain cDNA library. Seventeen clones positive using the beta-gal assay were further confirmed when cotransformed with pGBKT7/EPM2A and with pGBKT7 alone. Immunoprecipitation experiments are being performed to prove the in vivo interaction is specific. Since it has been shown that Cys-Ser mutation in the active site of other DSPs can trap the substrate such that the complex can be isolated by immunoprecipitation, we decided to perform our experiments using transfected Cos-cells with pcDNA3mycEPM2A and its Cys-Ser mutant. The cell lysate was immunoprecipitated using myc antibody and three bands of 40, 80 and 180KDa appeared in the wild-type and mutant but not in the vector alone. They are now being subjected to Mass Spectrometry. For GST pull-down experiments Cos-lysates were incubated with GST/EPM2A and the mutant GST/EPM2A Cys-Ser bound to the beads. After washing they were analyzed by SDS-PAGE. Four extra bands of 40, 80, 100, 120KDa were found only in the mutant GST/EPM2A C-S. A large scale experiment is underway to obtain a larger amount of protein for analysis.



**Direct Interactions of Fanconi Anemia Proteins in the Yeast Two Hybrid System.** *M.E. Matkovic, A.F. Foliás, A.M. Ward, J.A. Hejna, R.E. Moses.* Molecular and Medical Genetics, Oregon Health Sciences Univ., Portland, OR.

We used the yeast two-hybrid system to test for interactions between the Fanconi Anemia proteins FANCA, FANCC, FANCD2, FANCE, FANCF, and FANCG as well as FAZ-F. All proteins tested were expressed as fusions to the DNA-binding domain and/or activation domain of GAL4. Co-expression of two interacting proteins in this system leads to the transcriptional activation of three separate reporter genes: *HIS3*, *ADE2*, *lacZ*. We were able to confirm the known interactions between FANCG/FANCF, FANCG/FANCA, and FANCE/FANCC. We also uncovered a novel interaction between FANCG and FAZ-F. The interactions above have also been confirmed by co-immunoprecipitation of *in vitro* translated proteins. To date we have not found an interaction between FANCD2 and any of the Fanconi anemia (FA) proteins; nor did FANCD2 interact with itself. This is consistent with a role for FANCD2 downstream of the other FA proteins. Alternatively, an interaction between FANCD2 and other FA proteins may require either modification of FANCD2 or a third adapter protein.

**Identification of protein binding partners for the Prader-Willi syndrome candidate *necdin*.** *E.L. Organ, J.L. McCauley, J.S. Sutcliffe.* Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Prader-Willi syndrome (PWS) is a genomic disorder associated with paternal-specific deficiencies caused by interstitial deletions, unipaternal disomy or imprinting defects affecting human chromosome 15q11-q13. A candidate region of ~1.5 Mb containing multiple imprinted, paternally-expressed genes has been described in the proximal third of the larger deletion interval. One of the imprinted genes mapping to this region, *necdin* (*NDN*), has been suggested to encode a DNA binding protein that functions as part of a complex to mediate neural cell growth arrest. In order to further elucidate *NDN* function, we have sought to identify additional binding partners in brain. A full length *NDN* construct was employed to screen a mouse brain cDNA library in a yeast two-hybrid system that allows for rigorous suppression of background false positives induced by the native DNA binding activity of *NDN*. A regulator of G-protein signaling, a microtubule motor protein, a carboxypeptidase and a RING zinc finger protein were all identified from multiple clones in independent library screenings. Additional putative binding partners were identified; these include CDP-diacylglycerol synthase and a methyl-CpG binding protein. Ongoing studies seek to confirm the interactions between *NDN* and its putative binding partners in a mammalian cell culture system.

**Length Polymorphism of Thymidylate Synthase Enhancer Region is higher in human than in non-human Primates.** *Y-P. Zhang, H-R. Luo.* Lab. Cellular & Molec. Evol., Kunming Inst. Zool., CAS, Kunming, Yunnan 650223, People's Republic of China.

Thymidylate synthase (TYMS; TS; N5, N10-methylenetetrahydrofolate; dUMP C-methyltransferase; EC2.1.1.45) is an essential enzyme for the survival of cell proliferating. It has been reported that TS enhancer region (TSER) contains two, three, four, five or nine tandemly repeated copies of 28bp sequence in human. To understand the pattern of this length polymorphism in human, we sequenced 26 species of nonhuman primates and two monkey populations, including 32 samples of *Macaca mulatta* (*M.mul*) and 30 samples of *Rhinopithecus roxellanae* (*R.rox*). The TSER gene tree had been constructed using Neighbor-joining method with the *Mus musculus* TSER as outgroup. The phylogenetic relationship of these primates consisted with that of generally acknowledged. Length polymorphisms have also been found in the nonhuman primates, but at lower level. Most of the species had the homozygous length with 2 or 3 repeats of 28bp sequence (2/2 or 3/3) except for 4/4 in the *Macaca thibetana* and *Macaca assamensis*, and 2/3 in the *Presbytis phayrei* and *Mandrillus leucophaecus*. Contrary to the high polymorphism in human population, the 28bp repeat allele is conservative in *R.rox* population with genotype 2/2, and in the *M.mul* population with genotype 3/3. No nucleotide variation had been found between the TSER alleles in human except for the difference of the repeat number. Comparison of the repeats in each of the human TSER allele, the last one repeat of them contains a 2-bp insertion (CC) and a single base substitution (G®C), whereas the other repeats have identical 28-bp segment. However, the *Pan troglodytes* and *Gorilla gorilla* show nucleotide variation besides the length polymorphism both in the TSER and in the flanking region. Especially, the last repeat in the apes TSER contains a 7-bp (GCTCGCC) insertion in addition to the 2-base insertion, but it has no G/C substitution.

**Functional analysis of single-minded 2 (SIM2) gene located on human chromosome 21q22.2.** *Y. Shimizu<sup>1</sup>, A. Yamaki<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 of Health Sci, Hachioji, Tokyo, Japan; 2) Dept Mol Biol, Keio Univ Sch of Medicine, Tokyo, Japan.

Human SIM2 gene encodes a PAS family transcription factor and it has been postulated to be a candidate gene for the pathogenesis of mental retardation in Down syndrome. To understand the molecular mechanism of SIM2 gene expression, we have analyzed the promoter region using series of deletion constructs and point mutants fused with luciferase reporter gene and gel retardation assay. we found that c-myb like transcription factor appears to regulate the transcription of human SIM2 gene. Furthermore, we found another region to have a promoter activity located between nt-878 and -835 that is 454bp downstream from the cis-element for c-myb. To determine the nuclear translocation signal of SIM2 protein, we have generated fusion genes of full-length cDNA and deletion mutants of SIM2 with the green fluorescent protein (GFP). Transient expression analysis in HeLa cells revealed that bHLH-PAS and HST domains are not necessary and the region of codon 348 to 399 is sufficient for the nuclear localization of SIM2 protein. We have made a series of point mutants between codon367 and 382 where 8 basic amino acids exist. We found the most important amino acid moiety for nuclear translocalization is arginine of codon 367.

**Expression of the *slc26a3*, *slc9a3*, and *cftr* genes in rat intestine show response to glucocorticoid.** *S.P. Mäkelä<sup>1</sup>, K. Kaunisto<sup>2</sup>, P. Höglund<sup>1</sup>, J. Kere<sup>1,3</sup>*. 1) Dept Medical Genetics, Univ of Helsinki, Finland; 2) Dept Anat and Cell Biol, Univ of Oulu, Finland; 3) Finnish Genome Center, Univ of Helsinki, Finland.

The SLC26A3 gene (alias CLD or DRA) encodes an intestinal epithelial Cl/HCO<sub>3</sub> exchanger that is defective in autosomally recessively inherited congenital chloride diarrhea (CLD, OMIM 214700). The major clinical feature of CLD is severe watery diarrhea with a high concentration of chloride. On the basis of diarrheal phenotype occurring in CLD patients who lack the functional gene, SLC26A3 has been suggested to be the major Cl/HCO<sub>3</sub> exchanger involved in coupled electronegative NaCl absorption in colon. The major exchanger involved in Na<sup>+</sup> absorption has been proposed to be the Na/H exchanger SLC9A3 (alias NHE3), causing diarrheal phenotype in mice if defective. In colon, the major secretor of Cl<sup>-</sup> to intestinal lumen is CFTR that has also been shown to regulate other ion transporters. We have used real-time quantitative RT-PCR using Taqman to investigate the expression profiles of these three intestinal transport genes in different segments of rat intestine. Because glucocorticoid treatment has been shown to reduce diarrhea in a variety of diarrheal states, we have also investigated the effect of glucocorticoid stimulation on the levels of these transcripts in vivo.

As expected, the basal mRNA expression levels of both *slc26a3* and *slc9a3* increased in the distal intestine and were highest in proximal colon. While the expression of *slc26a3* remained high also in distal colon, the expression of *slc9a3* decreased. The basal expression of *cftr* was constant in all regions of intestine studied. The main effect of glucocorticoid stimulation was observed in proximal colon where marked increase in the expression of all three genes was detected. *Cftr* showed response to glucocorticoid in all segments of intestine studied, whereas no clear effect could be detected with *slc26a3* and *slc9a3* in jejunum or with *slc9a3* in distal colon. This study clarifies further the intestinal electrolyte transport physiology and the molecular pathology of CLD. It also elucidates molecular mechanisms by which glucocorticoids regulate fluid and electrolyte homeostasis.

**From Diseases of Anion Transport to New Members of the SLC26 Anion Transporter Family.** *M.M. Kujala<sup>1</sup>, H. Lohi<sup>1</sup>, S.P. Mäkelä<sup>1</sup>, E. Lehtonen<sup>2</sup>, J. Kere<sup>1,3</sup>.* 1) Department of Medical Genetics, University of Helsinki, Finland; 2) Department of Pathology, University of Helsinki, Finland; 3) Finnish Genome Center, University of Helsinki, Finland.

The previous sulfate transporter gene family has recently been newly delineated in human as a second distinct family of anion transporters, SLC26, in addition to the classical SLC4 (or AE) family. The three first known human SLC26 genes are all associated with dissimilar recessively inherited diseases. The diastrophic dysplasia sulfate transporter (DTDST) gene (SLC26A2) causes diseases of the bones and cartilage when mutated. Defects in the down regulated in adenoma (DRA) gene (SLC26A3) produce congenital chloride diarrhea (CLD). Pendred syndrome (childhood deafness and goiter) is caused by mutations in the Pendrin (PDS) gene (SLC26A4). Members of the SLC26 family are structurally well conserved and can transport with different specificities at least the chloride, iodine, bicarbonate, hydroxyl, sulfate and oxalate anions.

We report the expansion of the human SLC26 family with seven new members (SLC26A1 and SLC26A6-A11) in chromosomes 1, 3, 4, 6, 8, 12 and 17. The new proteins are structurally highly homologous to the previous anion transporters, suggesting that they as well function as anion transporters. The new members of the SLC26 family are expressed in variety of different tissues, most of them in a highly tissue-specific manner. We are in the process of characterizing their tissue and cell type distributions in detail. For example, SLC26A8 is expressed specifically in male germ cells in the testis, suggesting that specific anion exchange may be important for these cells. The functional characterization of the novel members of this tissue-specific gene family may provide new insights to anion transport physiology in different organs.

**A new retinal gene, RP1L1, which encodes a protein similar to RP1 and which has unusual polymorphic variation.** *L.S. Sullivan<sup>1</sup>, S.J. Bowne<sup>1</sup>, K.A. Malone<sup>1</sup>, J.R. Heckenlively<sup>2</sup>, S.P. Daiger<sup>1</sup> and The RP1 Consortium.* 1) Human Genetics Ctr, Univ Texas, Houston, TX; 2) Jules Stein Eye Institute, UCLA, Los Angeles, CA.

The RP1 gene causes approximately 6-10% of autosomal dominant retinitis pigmentosa cases. The protein localizes to the connecting cilia of rod and cone photoreceptors and may be involved in protein transport to the outer segment. The doublecortin domain of RP1 probably interacts with microtubules of the cilium but the function of the rest of the protein remains to be determined.

We have identified a novel retinal gene, RP1L1, with a very similar genomic structure and significant sequence similarity to RP1. This gene codes for a protein 2,414 amino acids in length (compared to 2,156 aa for RP1) and has 32% identity and 53% similarity to RP1 over the first 350 amino acids. This region of similarity includes and extends beyond the doublecortin domain and suggests that RP1L1 is RP1's closest protein relative. Preliminary evidence suggests that RP1L1 expression is limited to the retina. The presence of a conserved doublecortin domain implies that RP1L1 may be another microtubule associated protein and might perform a parallel function to RP1. There is also some weak sequence similarity to RPGR, the gene responsible for the RP3 form of retinitis pigmentosa. RPGR has also been recently localized to the photoreceptor connecting cilia.

The middle of the putative RP1L1 protein is highly repetitive and contains a tandemly repeated 16 aa sequence which appears to be polymorphic. We have identified at least three RP1L1 alleles which contain 0-4 of these repeats, embedded within a run of imperfect repeats. The only other reported occurrence of this kind of tandemly repeated polymorphic sequence (within a gene) is in the dopamine D4 receptor.

Because of the similarity of RP1L1 to RP1 we are investigating the possibility that RP1L1 might cause inherited retinal degeneration. Additionally, it might be involved in a parallel pathway which could potentially compensate for the loss of RP1 in patients with the RP1 form of adRP.

**Structure and localization of the *GFRA4* locus and investigation of the gene in human thyroid cancer.** *J.B. Vanhorne, K.J. Harrison, B. Thomas, S.A.M. Taylor, L.M. Mulligan.* Department Pathology, Queen's University, Kingston, ON, Canada.

Glial cell line-derived neurotrophic factor (GDNF) family receptors a (GFRas) are cell surface bound co-receptors required for activation of the RET receptor tyrosine kinase by members of the GDNF family. GFRa family members have strong sequence and structural homology. We have shown that their gene structures are also conserved. The recently identified GFRa-4 is the primary co-receptor for binding of persephin to RET. GFRa-4 differs structurally from other GFRa family members, lacking one of 3 cysteine rich domains thought to confer secondary structure. To understand the relationship of GFRa-4 and other GFRa family members, we investigated the structure and localization of the *GFRA4* gene. We mapped the *GFRA4* locus by FISH to chromosome 20p13. Comparison of *GFRA4* cDNA and genomic sequences for this region indicated that *GFRA4* contains 6 coding exons spanning approximately 4 kb of genomic DNA. Consistent with previously reported protein structure differences, *GFRA4* has an interstitial deletion relative to other members of the *GFRA* gene family, resulting in absence of at least 2 exons conserved in all other family members. GFRa-4 is strongly expressed in thyroid and has been predicted to act as the primary RET co-receptor in this tissue. As activating mutations of *RET* are associated with familial thyroid tumors in multiple endocrine neoplasia type 2 (MEN 2) and are found in a subset of sporadic medullary thyroid carcinoma (MTC), we postulated that mutations or variants of *GFRA4* could alter the level of RET activation occurring in these cells and might also contribute to this disease phenotype. We have investigated a group of MEN 2 cases without detectable *RET* mutations and a panel of individuals with apparently sporadic MTC for mutations or variants of *GFRA4*. We identified polymorphic variants in exons 1, 2, 5 and 6 of *GFRA4* in both normal controls and patient samples. We did not detect any difference in frequency of these variants between our populations. Our data suggest that mutations of *GFRA4* are not a common contributing factor to heritable or sporadic thyroid neoplasia.



**Spatial and temporal control of the human ADH gene complexes in BAC transgenic mice.** *J.S. Su<sup>1</sup>, K.M. Chao<sup>2</sup>, S.F. Tsai<sup>1,3</sup>, T.F. Tsai<sup>1,2</sup>.* 1) Institute of genetics, National Yang-Ming University, Taipei, Taiwan, ROC; 2) Department of Life Science, National Yang-Ming University, Taipei, Taiwan, ROC; 3) Division of Molecular and Genomic Medicine, National Health Research Institutes, Taipei, Taiwan, ROC.

Human 4q21-24 region was previously shown to bear a tumor suppressor gene for human liver cancer. We have conducted genomic sequencing for this region and the syntenic mouse chromosome 3 region. Seven human ADH genes and four mouse Adh genes were identified from the compiled sequences. The PipMaker program (Schwartz, et al., 2000) predicted several conserved noncoding sequences (CNS) for the human and mouse alcohol dehydrogenase (ADH) gene complexes. To identify functional significance of these conserved sequences, in terms of spatial and temporal control of ADH gene expression, we have prepared BAC transgenic mice using sequenced BAC DNA encompassing different region of the CNS. Human ADH gene expression was analyzed in fetal and adult transgenic mouse livers to demonstrate tissue and developmental specificity. The results indicate that, despite the difference in Class I ADH gene numbers, three class I human ADH genes were expressed in a manner similar to the patterns observed in human tissues. Thus, a critical segment regulating the entire class I ADH gene expression is mapped to the conserved noncoding sequences between human ADH3 and ADH7.

**Characterization of a novel gene within the Smith-Magenis syndrome critical interval.** *R.E. Lucas*<sup>1</sup>, *C.N. Vlangos*<sup>1</sup>, *T. Newton*<sup>2</sup>, *S.H. Elsea*<sup>1,2,3</sup>. 1) Genetics Graduate Program, Michigan State University, East Lansing, MI 48824; 2) Department of Zoology, Michigan State University, East Lansing, MI 48824; 3) Department of Pediatrics/Human Development, Michigan State University, East Lansing, MI 48824.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation syndrome and possible contiguous gene deletion syndrome associated with an interstitial deletion of human chromosome 17p11.2. SMS has a characteristic behavioral and physical phenotype and is estimated to occur in approximately 1 in every 25,000 births. Towards a greater understanding of the molecular basis of SMS, we recently generated an overlapping and contiguous transcription map of the SMS critical interval, linking the proximal 17p11.2 region near the SMS-REPM and the distal region near D17S740. Within the SMS critical interval, we identified 16 known genes, 12 ESTs, and 6 genomic markers. In this study, we have focused on EST DKFZp434A139Q2, which represents the 3' end of the novel gene, retinoic acid induced 1 (*RAII*). Northern analysis reveals that *RAII* is expressed in all adult and fetal tissues studied. Through hybridization to digested BACs and PACs within the SMS critical interval transcription map and human genome project sequencing analysis, we determined that *RAII* maps to the central region of the SMS critical interval, adjacent to *SREBF1*. A gene or genes in this region of the SMS critical interval may play an important role in craniofacial development. In order to gain a greater understanding of the cellular role of *RAII*, we isolated a full-length cDNA clone to create a full-length cDNA-green fluorescent protein construct which will be transfected into mammalian cell lines and used to determine the cellular localization of the novel *RAII* protein. We have also identified mouse and human genomic clone for *RAII* and determined putative intron/exon boundaries. A mouse knockout construct will be developed so that we may gain information about the biochemical processes in which this novel protein may be involved and ascertain the effect of haploinsufficiency.

**Molecular cloning of a novel putative murine mitochondrial transporter.** *F.Y. Li<sup>1</sup>, B. Leibiger<sup>2</sup>, I. Leibiger<sup>2</sup>, C. Larsson<sup>1</sup>*. 1) Department of Molecular Medicine, CMM, L8:01, Karolinska Hospital, SE-171 76 Stockholm, Sweden; 2) Department of Molecular Medicine, The Rolf Luft Center for Diabetes Research L3, Karolinska Hospital, SE-171 76, Stockholm, Sweden.

We report here a novel murine gene, *Mrs3/4*, encoding a putative mitochondrial transporter homologous to the yeast mitochondrial RNA splicing protein 3 and 4 (yMRS3&4) and its human counterpart hMRS3/4. By analyses of radiation hybrids *Mrs3/4* was mapped to mouse chromosome 19, in a region which is syntenic to human chromosome 10q24 where the human gene is located. Structural analysis shows that these proteins belong to the mitochondrial carrier family (MCF), which is characterized by three repeats with two transmembrane domains in each repeat. The murine *Mrs3/4* gene has two splicing forms similar to the human homologue. The long form corresponds to the 341-amino acids (aa) protein with six transmembrane domains and the short form to the 177-aa protein with three transmembrane domains. Both forms have well-conserved signature sequences of MCF. Targeting experiments showed that both forms have mitochondrial localization. Northern blot analyses showed that *Mrs3/4* is ubiquitously expressed as a 1.8 kb transcript with a relative abundance in heart, liver, kidney and testis. In conclusion, the reported *Mrs3/4* gene shows a strong conservation, mitochondrial protein localization and a ubiquitous expression which suggest that it has an important role in mammalian cells most likely as an ion transporter across the mitochondrial inner membrane.

**The Mammalian Fem1c Genes: New Members of the Fem1 Gene Family.** *J.F. Maher, T. Ventura-Holman, S. Xiaohong.* Dept. of Medicine, University of Mississippi Medical Center and G.V.(Sonny) Montgomery Veterans Affairs Medical Center, Jackson, MS.

In the nematode *Caenorhabditis elegans* the fem-1 gene is in a genetic pathway that controls sex-determination. The product of this gene, FEM-1, appears to be involved in signal transduction between an extracellular signal, HER-1, and a transcription factor, TRA-1. Our laboratory has reported two homologs in the mouse, Fem1a and Fem1b. These two genes have highly related homologs in humans, designated FEM1A and FEM1B, respectively. We report here the characterization of a third member of this gene family, highly conserved between mouse and humans and designated Fem1c and FEM1C, respectively. At the protein level, mouse Fem1c and human FEM1C show > 99 % amino acid identity. FEM1C is most highly related to FEM1A. For example, with the encoded amino acid sequences, human FEM1C is 70% identical to human FEM1A, and 41% identical to human FEM1B (by comparison, human FEM1A and FEM1B share 37% amino acid identity). We have mapped the human FEM1C gene to chromosome 5 by FISH analysis, and this has now been verified by available Human Genome Project sequencing data. Northern Blot analysis shows some conservation of expression pattern in adult tissues between mouse and human genes, with message most abundant in testis in both organisms. The gene is also expressed in a mouse embryo Northern Blot of day 7 through day 17. Efforts are now underway to ascertain the biological functions of the human and mouse FEM1C genes. We present evidence that there is a Fem1c homolog in Teleost fish, suggesting a very ancient origin of its divergence from the Fem1a / Fem1b family members.

***hOAT1*, a novel organic anion transporter in kidney.** D. Prawitt<sup>1</sup>, A. Bahn<sup>2</sup>, T. Enklaar<sup>1</sup>, S. Fees<sup>1</sup>, H. Hauser<sup>1</sup>, L. Brixel<sup>1</sup>, C. Spangenberg<sup>1</sup>, F. Quondamatteo<sup>2</sup>, A. Hillemann<sup>2</sup>, R. Herken<sup>2</sup>, G. Burckhardt<sup>2</sup>, B.U. Zabel<sup>1</sup>. 1) Molecular Genetics Lab, Children's Hosp/ Univ Mainz, Mainz, Germany; 2) Center for Physiology and Pathophysiology, Goettingen, Germany.

The recently cloned organic anion transporter 1 (OAT1) belongs to the OAT subfamily of sugar porter proteins which all display remarkable structural homologies. They are expressed in the proximal tubule cells of the kidney and mediate the excretion of a variety of structurally unrelated substances including PAH, many drugs and xenobiotics. To elucidate the genomic structure we isolated a genomic *hOAT1* PAC clone and determined the exon-intron organization of the *hOAT1* gene. It encodes a 12 transmembrane protein with a large extracellular loop between the first two transmembrane domains. RT-PCR analysis on human kidney samples led to the detection of two new splice variants (*hOAT1-3* and *hOAT1-4*) with a 132 bp inframe deletion of the ORF as part of exon 9. Gene mapping experiments placed the *hOAT1* gene on chromosomal region 11q13.1-q13.2. Further elucidation of the OAT1 function and its implication in cellular processes was performed with the human gene (*hOAT1*) and the rabbit orthologue (*rbOAT1*). Transcription was not only observed in kidney and liver, but also in non secreting tissues like muscle and eye. In situ hybridization of rabbit eye sections with the renal *rbOAT1* as a probe revealed positive signals in the endothelium of inner blood vessels, the ganglion cells and several neurons in the bipolar cell layer of the retina. This is the first description of an organic anion transporter in neuron cells and it is tempting to speculate about *hOAT1* playing a role in transport processes of neurotransmitter metabolites in the visual system. *hOAT1* might therefore be involved in diseases where accumulation of these substances in the central nervous system is of critical importance.

**Functional studies of a recurrent mutation in Notch3 in CADASIL.** *T. Haritunians<sup>1</sup>, T. Chow<sup>2</sup>, R. P.J. De Lange<sup>3</sup>, N. Dorrani<sup>1</sup>, D. M. St. Clair<sup>3</sup>, N.C. Schanen<sup>1</sup>.* 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Dept Neurology, Univ California, Los Angeles, Los Angeles, CA; 3) Dept Mental Health, Univ Aberdeen, Aberdeen, UK.

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is an inherited vascular dementia, characterized pathologically by a loss of vascular smooth muscle cells in the tunica media of small penetrating arterioles in the brain. The ultimate destruction of these vessels leads to recurrent ischemic strokes in the deep white matter, resulting in progressive dementia and neurological deficits in relatively young adults that lack the common risk factors for stroke. It has been identified that most CADASIL patients carry missense mutations in the NOTCH3 gene. Notch3 is part of a larger gene family of evolutionarily conserved cell surface receptors that plays a role in cell fate determination during development. Here, we have identified a new CADASIL family that carries a C185R mutation in exon 4 of the NOTCH3 gene, which results in a Cysteine substitution in the fourth epidermal growth factor (EGF)-like repeat of the extracellular domain. The EGF-like repeats have been proposed to be important for receptor binding to cell surface bound ligands, Delta and Jagged. Using site-directed mutagenesis, we have created the analogous mutation (C187R) in rat Notch3. To assess the effect of this mutation on Notch3 function, the mutant protein was transiently expressed in 293T cells. Cell surface expression of the protein was confirmed and its ability to bind a novel soluble ligand, Delta-1-Fc, was characterized by immunofluorescence, in comparison to wild type Notch3. These studies will evaluate whether the CADASIL mutations interfere with ligand binding or with downstream signal transduction. Supported by AHA 9950030N.

**Late Fifth Instar Larval Stage U1 and U2 snRNA Isoforms from the Silk gland of the silkworm, *Bombyx mori*.**

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U1 and U2 snRNA isoforms have been isolated and sequenced from U-specific cDNA libraries generated from the silk gland of the late fifth instar larval stage of the silkworm *Bombyx mori*. Due to the highly specialized nature of the silk gland and its 200,000-fold increase in DNA, it is an ideal candidate for gene expression studies. RT-PCR, cloning, automated sequencing (ABI 377) and secondary structure computer modeling analysis (Mike Zukers RNAdraw program) were utilized to examine the nucleic acid sequence of the full-length U-snRNAs. Five U1 and seven U2 snRNA variants were obtained from screening over 200 clones from their respective cDNA libraries. These isoforms exhibit 1) compensatory base changes (transitions/transversions), 2) conservation of secondary structure, 3) highly conserved protein binding sites (e.g. U1 70K, U2A and Sm binding site), 4) changes in localized regions in moderately conserved sites (e.g. base of loops) and 5) base-pairing generating more stable hairpin structures. The U1 and U2 snRNA sequences are highly conserved across species. Small nuclear RNA molecules have also been shown to be developmentally linked (i.e. mouse U1-specific fetal and adult forms) but none have been correlated to the insect larval stage. The focus of current studies is the examination of U-specific cDNA libraries generated from the silk gland of early and middle fifth instar larval stages.

**Autosomal Dominant Polycystic Kidney Disease Type I: Genes and Pseudogenes.** *J. Horst<sup>1</sup>, A. Markoff<sup>2</sup>, B. Dworniczak<sup>1</sup>, N. Bogdanova<sup>1</sup>*. 1) Inst Human Genetics, WWU Muenster, Muenster, Germany; 2) Institut fr Medizinische Biochemie, Univ. Mnster; Mnster, Germany.

One of the most common monogenic inherited disorders in men, the autosomal dominant polycystic kidney disease (ADPKD), with an incidence of approximately 1 in 1000, is mainly characterized by development of fluid-filled cysts in both kidneys, resulting in most cases in kidney failure. In affected individuals of European descent, mutations in the PKD1 gene, located on 16p13.3 are the most common cause for the disease and account for up to 85% of the cases. The PKD1 gene extends over ~52 kb of genomic DNA and contains 46 exons encoded by a 14 kb transcript. A large part of the gene, extending from exon 1 to the first 87 bp of exon 33, is duplicated in at least three homologous genes (HG), which are located on chromosome 16p13.1 and share approximately 95-97% homology with the PKD1 gene, thus heavily obstructing the mutation analysis of PKD1. Two of these genes have been recently covered in a large sequencing work on chromosome 16. It has been however not known for years if homologous to PKD1 genes code for functional polypeptides. We have identified four more homologues to PKD1 which are different from the previously published sequence by employing PCR screening of a bacterial artificial chromosome (BAC) DNA library and BLAST homology searches in publicly available data bases. Deciphering the structural differences between HG and PKD1 allows for differential analysis of those genes. Assaying their transcripts in the model cell line T98G shows that HG are not translated. Taken together with the sequence information about the genes, these data show that homologues to PKD1 are pseudogenes. This is the first work on PKD1 homologous genes to demonstrate that HG belong to a PKD1 pseudogene family, the members of which emerged at about the same time in the course of molecular evolution. In addition, the precise structural characterization of the PKD1 homologues allows to create better reagents for conventional mutation analysis of the duplicated part of the PKD1 gene and to review the mutation data of the duplicated part of the gene obtained by others.



**Lack of copies of the DAZ gene cluster in normal and infertile males without apparent Yq microdeletions. L.**

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About 10% of infertile men show microdeletions of the Y chromosome long arm (Yq) involving three different loci, AZFa, AZFb, AZFc. Deleted in Azoospermia (DAZ) gene is the major candidate for male infertility, being involved in about 60-70% of all microdeletions, and nearly in 100% of deletions involving AZFc. This gene maps within subinterval 6D of the Y chromosome with multiple copies. The typical PCR approach for the detection of Yq microdeletions is able to disclose only deletions removing all the DAZ copies, but not those leaving as few as one copy of the gene. Thus, it is not known whether or not partial deletions of DAZ can be related to male infertility. Based on the presence of intronic single base changes, four copies of DAZ (DAZ1-4) are detectable by PCR amplification of 3 intragenic STSs followed by specific endonuclease digestion. Using this approach, we analyzed 42 infertile patients without apparent microdeletion of the Y chromosome, and 67 fertile controls. Three STS of the DAZ gene (sY581, sY586, SY587) were amplified by PCR and digested with *Sau3A*, *Taq I*, and *Dra I*, respectively. Absence of the band corresponding to the DAZ2 gene was detected in 13 infertile patients (30.9%) and in 10 controls (14.9%). Thus, the absence of this copy of the DAZ cluster appear compatible with normal fertility, being present in about 15% of controls. However, the incidence of this condition in infertile patients was two times higher than in controls. This could mean that subjects lacking this copy of DAZ could be more prone to other genetic and environmental factor in turn responsible of the infertility. On the other hand, simultaneous absence of DAZ1 and DAZ4 were detected in two infertile patients (4.7%), and other two had absence of DAZ2 and DAZ3. No control subject showed this condition, suggesting that this feature could be a cause of male infertility in patients not showing apparent Yq deletions.

**Identification of a novel human cytokine gene in the interleukin gene cluster on chromosome 2q12-14. J.T.**

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Genes residing in the interleukin cluster on chromosome 2q12-14, including interleukin-1 alpha (IL1A), interleukin-1 beta (IL1B) and interleukin-1 receptor antagonist (gene symbol IL1RN and protein symbol IL1Ra), are members of a family of IL-1 cytokines that play an important role in mediating the inflammatory response. We have identified a novel IL-1 like gene, designated IL1F10. Initially BLAST searches of GENBANK's high throughput genome sequence using known cytokine amino acid sequence identified 2 possible exons. Extensive cDNA cloning including 5' and 3' RACE was needed to identify the entire IL1F10 gene encoded by 5 exons spanning over 7.8 kb of genomic DNA. The 1008 bp IL1F10 cDNA encodes a 152 amino acid protein that shares 41 and 43% amino acid identity with human IL1Ra and FIL1 delta (Smith et al., J. Biol. Chem. 275:1169-1175 (2000)), respectively. IL1F10 maps to human chromosome 2, between FIL1 delta and the IL1RN. In addition to a TATA box, this gene contains a putative C/EBPb transcription factor binding site (-120 to -107) within the promoter similar to the promoter of the secreted form of IL1Ra. IL1F10 shares sequence characteristics of the IL1Ra family including, key amino acid consensus sequences and a similar genomic structure. Pairwise global alignment and calculation of evolutionary relatedness suggests that IL1F10 is a common ancestor from which other "IL-1 receptor antagonist-like" homologs arose through gene duplication. In multi-tissue cDNA panels, low-level expression was evident in non-immune tissues such as human heart and placenta. Consistent with a possible immunological role, this novel transcript was present in highest levels in fetal liver, spleen, thymus and tonsil (listed in level of expression from lowest to highest). The expression in a variety of immune tissues and similarity to IL1Ra suggest a role of IL1F10 in the inflammatory response.

**Analysis of the LMX1B promoter in human fibroblasts.** *J.A. Dunston, J.D. Hamlington, I. McIntosh.* McKusick Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Nail Patella Syndrome (NPS) is the result of heterozygous loss of function mutations in the LMX1B gene. This gene encodes a LIM-homeodomain transcription factor that is required for development of the limbs, eyes, kidneys and portions of the CNS. In this study, we identified the transcription start site and localized promoter elements using a luciferase assay reporter system. The transcription start site was mapped using primer extension and 5' RACE with RNA from HEK-293 cells as template. A 3.5 kb fragment containing the transcription start site was cloned from BAC 265A2, sequenced, and inserted into the luciferase vector (pGL3 basic). This fragment, which contains 2.7 kb of 5' flanking sequence and 0.8 kb of 5'UTR sequence, had significant promoter activity in human fibroblasts. Deletion of 5' sequence to -442 had little effect on reporter activity. Additional deletion to -379 caused a significant increase in reporter activity suggesting the presence of a repressor between -442 and -379. Deletion of 3' sequence to +460 caused a decrease in reporter activity indicating that sequence elements within the 5'UTR are capable of increasing the level of transcription.

**Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2 encoded by ABCG5 and ABCG8 respectively.** *K. Lu<sup>1</sup>, M.H Lee<sup>1</sup>, S. Hazard<sup>2</sup>, A. Brooks-Wilson<sup>3</sup>, G. Salen<sup>4</sup>, M. Dean<sup>5</sup>, A. Srivastava<sup>6</sup>, S.B. Patel<sup>1</sup>.* 1) Division of Endocrinology, Diabetes and Medical Genetics, Medical University of South Carolina, Charleston, SC; 2) BioMolecular Computing Resource, Medical University of South Carolina, Charleston, SC; 3) Xenon Genetics Inc., Suite 100, 2387 East Mall, Vancouver, BC, V6T 1Z3 Canada; 4) Division of Gastroenterology, University of Medicine and Dentistry New Jersey, Newark, NJ 07018; 5) Laboratory of Genomic Diversity, National Cancer Institute-Frederick, Frederick, MD 21702; 6) J. C. Self Research Institute of Human Genetics, Greenwood Genetics Center, Greenwood, SC 29646.

Sitosterolemia (MIM 210250) is a rare autosomal recessive disorder, characterized by intestinal hyperabsorption of all sterols, including cholesterol, plant and shellfish sterols and an impaired ability to excrete sterols into bile. Patients with this disease have expanded body pools of cholesterol, very elevated plasma plant sterol species and frequently develop tendon and tuberous xanthomas, accelerated atherosclerosis, and premature coronary artery disease. We previously mapped the STSL locus to human chromosome 2p21. Recently, we reported that a novel member of the ABC transporter family, named sterolin-1 and encoded by ABCG5, is mutated in 9 unrelated sitosterolemia families. In the remaining 25 families, no mutations in sterolin-1 could be identified. We identified another ABC transporter, located less than 400 bp upstream of sterolin-1, in the opposite orientation. Mutational analyses revealed that this highly homologous protein, termed sterolin-2, encoded by ABCG8, is mutated in the remaining pedigrees. Thus, two highly homologous genes, located in a head-to-head configuration on chromosome 2p21, are involved in causing sitosterolemia. These studies indicate that both sterolin-1 and sterolin-2 are indispensable for the regulation of sterol absorption and excretion. Identification of sterolin-1 and sterolin-2 as critical players in the regulation of dietary sterol absorption and excretion identifies a new pathway of sterol transport.

**Genomic structure of the human p22 dynactin light chain (DCTN3) and comparative mapping to rat**

**chromosome 5.** *D.R. Mills<sup>1</sup>, J. Pepperell<sup>2</sup>, U. Tantravahi<sup>2</sup>, M. Shiozawa<sup>3</sup>, A. Matsuzaki<sup>3</sup>, A.E. Kwitek-Black<sup>3</sup>, H.J.*

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The gene for the p22 dynactin light chain (DCTN3) was identified using positional cloning methods and localized to human 9p13 by radiation hybrid mapping. The DCTN3 protein has been demonstrated by other investigators to interact with the dynein microtubulebased motor complex, localize to kinetochores, and participate in cell cycle progression and cytokinesis. Human BAC clones were identified that contained the DCTN3 gene. Fluorescence in situ hybridization mapping confirmed that DCTN3 localized to human chromosome 9p13. The genomic organization of DCTN3 was characterized by sequencing longrange interexon PCR products using primers derived from the DCTN3 mRNA (GenBank accession number AF082513). The DCTN3 gene consists of 7 exons and 6 introns and spans approximately 6.9 kb. Introns 1, 5 and 6 are in phase 0, and introns 2, 3 and 4 are in phase 1. A neutral (CT) single nucleotide polymorphism (SNP) was identified at nucleotide position 34 of exon 1. Single strand conformation polymorphism (SSCP) screening of 100 random individuals demonstrated a low 2% frequency for this SNP. While comparative synteny is well established between human 9p13 and mouse 4, the relationship between human and rat is less well characterized. Human DCTN3 is flanked by the genes for GALT and CNTFR. We have localized the rat homologs of these three genes to a common region of rat chromosome 5 by fluorescence in situ hybridization mapping.

**Identification of New Genes from the Pericentromeric 7.4-Mb Region of Human Chromosome 22.** *T. Sasaki, A. Shiohama, K. Hosono, Y. Matsushita, S. Minoshima, A. Asakawa, J. Kudoh, A. Shintani, K. Shibuya, N. Aoki, S. Mitsuyama, K. Kawasaki, N. Shimizu.* Dept. Mol. Biol., Keio Univ., Tokyo, Japan.

The chromosome 22 sequencing consortium including us finished genomic sequencing of chromosome 22q (33.4 Mb) as the first human chromosome (*Nature* **402**:489-495, 1999), in which 545 genes (including 298 related and predicted gene) were reported. Since then, we have been carrying out further analysis of the pericentromeric 7.4-Mb sequence in combination of the manual evaluation of the computer output and experimental isolation of cDNAs. The analyzed regions include: the CES (cat eye syndrome) region (2.2 Mb), the DGS (DiGeorge syndrome) region (1.5 Mb), the BCRL2-GNAZ region (2 Mb), and the BCR-IGLL region (1.7 Mb). As a result, we have found 11 new genes including CESK1 from the CES region, C22ORF12 from the DGS region, LUK1 and LUK2 from the BCRL2-GNAZ region, C22ORF13, C22ORF14, SLC2A11 (GLUT11), ZNFLL1, C22ORF15, C22ORF16, and PNEC9 from the BCR-IGLL region. Here, we report the initial characterization of some of these new genes. CESK1 is an active gene located at the most centromeric on 22q and it consists of single exon. The isolated cDNA of CESK1 is 2.4-kb long, and has 1674-bp coding sequence encoding a protein of 557 amino acids. The deduced amino acid sequence showed a significant homology to human T-complex protein-1 theta (32% identity and 56% homology), which is known to have a chaperon-like activity. Northern blot analysis showed the CESK1 expression is limited in testis. However, PCR analysis using various tissue derived cDNAs showed amplified products in fetal brain as well as testis. GLUT11 (SLC2A11) has a significant homology (51%;) to a known glucose transporter GLUT5. It consists of 12 exons spanning 25-kb long genomic sequence and locates between SMARCB1 and MIF. The comparison with the other members of GLUT family showed that GLUT11 conserves the GRR/K motif located at right after transmembrane helices 2 and 8. The cDNA cloning results indicated the presence of three distinct exon-1s. PCR analysis using multi tissue-derived cDNA panels (MTC) confirmed the differential expression from each exon 1. Characterization of other genes will be presented.

**Characterization of complete methylenetetrahydrofolate reductase (*MTHFR*) genomic region identifies clusters of transcriptional start sites and translation start sites of two protein isoforms.** P. Tran, D. Leclerc, M. Chan, A. Pai, P. Goyette, F. Hiou-Tim, Q. Wu, R. Milos, C. Artigas, R. Rozen. Human Gen, Biol, Pediatrics, McGill Univ, Montreal, PQ., Canada.

MTHFR is a folate-metabolizing enzyme that links folate interconversion with homocysteine metabolism and the methylation cycle. MTHFR converts 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a major carbon donor in homocysteine remethylation to methionine. Two polymorphisms cause mild MTHFR deficiency, a condition shown to occur in approximately 35% of North Americans and Europeans. This deficiency may alter risk for neural tube defects, vascular disease, pregnancy complications and certain cancers. While these factors underscore the importance of understanding how *MTHFR* is regulated, molecular studies have been limited to a 2.2kb human partial cDNA that encodes a 70kD isoform of MTHFR. Observed transcript sizes of approximately 7.5kb and 9.5kb and a major protein isoform of 77kD suggest that additional cDNA sequences remain to be identified. Using library screening, PCR-based strategies and homology searches with the NCBI databases, the 5' and 3' UTRs of *MTHFR* were characterized in human and in mouse. In both species, exon 1 was redefined to approximately 4.0kb in length and is alternatively spliced. Expression studies demonstrated that an important splicing event within exon 1 gives rise to a novel coding region that encodes the predominant peptide of 77kD. Using ribonuclease protection assay, clusters of transcriptional start sites were detected, alluding to the presence of multiple promoters. Multiple polyadenylation sites were located which create 3'UTR lengths of 0.2kb-5.0kb and of 0.6kb-4.0kb for human and for mouse mRNAs, respectively. Finally, the 5' and 3' termini of the *MTHFR* cDNA were found to overlap with the 5' terminus of a chloride ion channel gene (*CLCN-6*) and with the 3' terminus of a thus far unidentified gene, respectively, mapping *MTHFR* more finely on chromosome 1p36. These results suggest that *MTHFR* is intricately regulated at several levels. Studies of the promoters and functional analyses of the different protein isoforms are in progress and will be presented.

**Fibroblast Growth Factor Receptor 2 (FGFR2) genomic sequence and variations.** *W.A. Paznekas, R. Ingersoll, A. KieuNgoc Tran, S. Rowe, L. Kasch, A.F. Scott, E.W. Jabs.* Institute of Genetic Medicine, Johns Hopkins University School of Medicine.

Fibroblast growth factor receptors (FGFRs) play a role in morphogenesis, development, angiogenesis, wound healing, and tumorigenesis. Mutations in FGFR2 cause more than five craniosynostosis syndromes. The FGFR2 genomic structure is the largest of the FGFR family. We have refined and extended the genomic organization of the FGFR2 gene by sequencing more than 119 kb of PACs, cosmids, and PCR products and assembling a region of approximately 175 kb from electronic databases. Although the gene structure has been reported to include only 20 exons, we have verified the presence of at least 22 exons, some of which are alternatively spliced. The sizes of six exons differ from those reported previously. The sequence of 20 of the 21 introns has been completed. Interspersed repeat DNA families are relatively enriched in intron 4, but are clustered in intron 2 preventing completion of its sequencing. The nucleotide sequence 5' to the start codon in exon 2 has two regions of 97 percent identity to the mouse promoter region. Comparison of our sequence and those in the NCBI database detected more than 300 potential single nucleotide polymorphisms (SNPs). However, sequencing regions containing 52 of these potential SNPs verified only 14 in PCR products generated from 20 CEPH alleles. In contrast, direct sequencing of the CEPH DNAs revealed 21 other polymorphisms. Only one SNP was found in the 2,926 bp of coding sequence. Twenty-seven SNPs, two insertion polymorphisms and five microsatellite polymorphisms are contained in approximately 18 kb of non-coding sequence. This gives an average of one SNP for every 700 bp of non-coding sequence examined. This collection of SNP, insertion and repeat polymorphisms will aid future association studies between the FGFR2 gene and human disease and will enhance mutation detection.



**A shorter MMP-9 promoter characterizes a mouse strain susceptible to glomerulosclerosis.** *A. Fornoni, O. Lenz, Y. Wang, M. Potier, L.J. Striker, G.E. Striker.* Vascular Biology Institute, Univ Miami, Miami, FL.

The genetic background plays an important role in the development of human glomerulosclerosis (GS). We have developed mouse strains that are glomerulosclerosis-prone (ROP) or resistant (C57). GS results from an imbalance between extracellular matrix (ECM) synthesis and degradation, MMP-9 being one of the major enzymes involved in the regulation of ECM degradation. We have previously shown that mesangial cells (MC) from ROP mice express very low levels of MMP-9 in comparison to C57 MC. The aim of this study were to examine whether the MMP-9 promoter structure is responsible for the differences in MMP-9 expression between ROP and C57 MC, and whether those findings are applicable to humans.

DNA extracted from ROP and C57 MC as well as from kidney cortex and tails was analyzed by SSLP analysis of the d(CA)<sub>n</sub> repetitive region (-142 to -90). In addition, the MMP-9 promoter region between -1167 and +90 was sequenced. Both ROP and C57 MMP-9 promoters were cloned into a pGL3 basic vector and transfection experiments in both MC and in COS7 cells were performed before and after stimulation of protein kinase C (PKC) by PMA. SSLP analysis of the MMP-9 promoter was also performed on DNA of MC from patients with and without GS.

We found that the smaller number of d(CA) repeats in ROP MC (20 vs 24 in the C57 strain) was at least partially responsible for a lower MMP-9 expression. SSLP analysis from tails and kidney cortex confirmed the strain difference in MMP-9 promoter length. The shorter ROP MMP-9 promoter appeared to be functional, i.e. responsive to PKC stimulation, although MMP-9 expression in ROP MC never reached the level observed in C57 MC. MC isolated from a patient with GS had a shorter MMP-9 promoter than MC from a patient without GS.

In conclusion we found that the ROP strain is characterized by a decreased number of d(CA) repeats in the MMP-9 promoter that influences MMP-9 gene expression. Furthermore, the shorter MMP-9 promoter in MC isolated from a patient with GS suggests that the length of d(CA) repeats in the MMP-9 promoter may be a marker of susceptibility to GS.

**Most missense mutations in neural cell adhesion molecule L1 affect intracellular trafficking or ligand binding. E.**

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Mutations in the gene for neural cell adhesion molecule L1 underlie an X-linked neurological disorder that includes hydrocephalus, mental retardation, adducted thumbs and spasticity. About 40% of mutations described in families are missense. These are mostly unique and affect residues that are distributed primarily across the extracellular domains required for ligand binding. Assigning pathogenicity to missense mutations is only possible when functional effects are demonstrated. We have therefore examined the effects of 26 different missense mutations, affecting the extracellular domains of L1, on binding to homophilic (L1) and heterophilic (TAX-1) ligands as well as on intracellular trafficking. 23 out of 26 mutations result in a significant reduction in binding to one or more ligands or ability to reach the cell surface. For mutations that retard protein movement to the cell surface, peri-nuclear accumulation and retention within the endoplasmic reticulum is found. A correlation between morbidity and type of mutation is emerging, with mutations that affect key structural amino acids more likely (44%) to result in death under 1yr than those that affect surface properties of the molecule (13%). This may be due to the fact that key-residue mutations are more often found to affect intracellular processing and/or ligand binding.

**Clustering of ATM mutations in A-T patients with intermediate radiosensitivity.** *X. Sun<sup>1</sup>, M. Mitui<sup>1</sup>, H. Chun<sup>1</sup>, B. Crandall<sup>2</sup>, R.A. Gatti<sup>1</sup>.* 1) Department of Pathology, UCLA School of Medicine, Los Angeles, CA90095-1732; 2) Department of Pediatrics, UCLA School of Medicine, Los Angeles, CA.

One of the unique characteristics of cells from patients with ataxia-telangiectasia (A-T) is hypersensitivity to ionizing radiation. To date, more than 400 mutations have been identified in the ATM (A-T mutated) gene [<http://www.vwresearch.org/atm.htm>]. We report here a clustering of mutations in 10 patients (13 mutations) with an intermediate response in the colony survival assay (CSA) for radiosensitivity, as compared to the random distribution of ATM mutations in 102 A-T patients with characteristic radiosensitivity. The mean survival fraction (SF $\pm$ 1SD) for patients with A-T was 13.4 $\pm$ 7.8%, as compared to 50.1 $\pm$ 13.5% for normals. The response of these 10 A-T patients, who were typical in all other ways, fell within an intermediate, non-diagnostic range (SF=21-36%). Their ATM mutations tended to cluster within either the p53-binding domain, the RAD3 homology region, or the PI-3 kinase domain of the ATM gene. This observation invites speculation as to how such a genotype/phenotype correlation could arise when most A-T patients have little or no detectable ATM protein by western blotting. We hypothesize those small amounts of ATM protein may escape detection by conventional methods while still playing a significant role in the response to ionizing radiation.

**Characterization of PITX2 Rieger syndrome mutations in *C. elegans*.** R. Toro<sup>1</sup>, I. Saadi<sup>1</sup>, Y. Jin<sup>3</sup>, B.G. Condie<sup>4</sup>, A.F. Russo<sup>1,2</sup>. 1) Genetics Program; 2) Department of Physiology and Biophysics, University of Iowa, Iowa City, IA; 3) Department of Biology, University of California Santa Cruz, Santa Cruz, CA 95064; 4) Developmental Biology Program, Medical College of Georgia, Augusta, GA 30912.

Mutations in the PITX2 gene have been established to play a role in the autosomal dominant Rieger syndrome. In the nematode *C. elegans*, the PITX2 homologue unc30 has been shown to regulate the glutamic acid decarboxylase gene unc25. Glutamic acid decarboxylase (GAD) catalyzes the production of the neurotransmitter GABA. *C. elegans* mutants that lack unc30 have a 'shrinker' behavioral phenotype that is characterized by an abnormal response to head touch stimulus. Interestingly, mouse Pitx2 can rescue this phenotype. We have shown that unc30/unc25 relationship extends to mammals; PITX2 can regulate the glutamic acid decarboxylase (Gad1) promoter. PITX2 shows a 5-8 fold activation of a reporter gene driven by the Gad1 promoter in various cell culture lines. Testing the functional consequences of the PITX2 Rieger syndrome mutations, such as the W133Stop truncation and the dominant-negative K88E, yielded unexpected cell-specific results in co-transfection experiments. For instance, the W133Stop protein shows both activation and inhibition depending on the cell line. We plan to test these mutations in *C. elegans* in order to extend these results to an in vivo system. The effects of the Rieger mutations will be assayed by scoring both the behavioral phenotype and expression of an unc25 driven GFP reporter in both wild type and unc30 mutant worms.

**Cloning and characterization of a novel apoptosis-associated gene expressed during erythroid cell differentiation.**

*W. Aerbajinai, J.L. Miller.* Laboratory of Chemical Biology, NIDDK/NIH, Bethesda, MD.

In recent years, we have taken a genomics approach toward understanding the transcriptional basis of human erythropoiesis (<http://hembase.niddk.nih.gov/>). We report here the molecular cloning and characterization of a novel erythroid differentiation-related gene called EDRG9 that is located on the short arm of chromosome twelve. The primary transcript, EDRG9-L, was cloned from two expressed sequence tags catalogued within our genomic database. The EDRG9-L mRNA encodes a putative protein of 1078 amino acids with a C1q-like globular domain at the COOH terminus. We also characterized an alternative transcript lacking exon 5 (EDRG9-S) that encodes a significantly smaller protein lacking the C1q domain. Northern blotting revealed expression of EDRG9 in multiple human tissues with strong signals found only in bone marrow and the fetal liver. RT-PCR analyses of mRNA obtained from primary erythroid cells at defined developmental stages were performed to determine the transcriptional profile of EDRG9 during erythropoiesis. Increased expression was noted among cells undergoing the proerythroblast-basophilic normoblast transition associated with global nuclear condensation and reduced cell proliferation during terminal differentiation. This expression pattern was shared by both EDRG9 isoforms and is similar to the erythroid transcriptional profile of genes associated with regulation and exit from the cell cycle. Plasmids encoding green fluorescent protein (GFP) tagged EDRG9 were also constructed and transfected into CHO cells for cell localization and functional assays. Expression of the GFP-tagged EDRG9-L transcript was localized to the cytoplasmic compartment, and no specific changes in the cellular phenotype have been identified to date. In contrast, overexpression of GFP-tagged EDRG9-S resulted in apoptosis of transfected CHO cells within 24-48 hours confirmed by TUNEL assay. Fluorescence microscopy studies demonstrated GFP-EDRG9-S localized with aggregating mitochondria. We propose that expression of the EDRG9 gene may be important in regulating proliferation and apoptosis during terminal erythroid differentiation.

**A novel retinoic acid-sensitive gene isolated by an induction gene trap approach.** *M. Schlicker<sup>1</sup>, O. Batista<sup>1</sup>, W. Wurst<sup>2</sup>, I. Hansmann<sup>1</sup>.* 1) Institut für Humangenetik, Martin-Luther-Universität, Halle, Germany; 2) Molekulare Neurogenetik, GSF-Forschungszentrum für Umwelt und Gesundheit, Neuherberg, Germany.

In man and mammals prenatal exposure to the teratogens retinoic acid (RA) and/or retinoids (RX) results in characteristic congenital anomalies including cardiac and craniofacial malformations. Incidence and pattern of the malformations apparently depends on dose and stage of exposure. Yet, the respective molecular mechanisms as well as the genes involved are more or less unknown. RA/RX signalling is mediated by nuclear receptors that activate target genes containing RA-binding elements (RAREs). Based on a retinoic acid-induced gene trap approach (Forrester et al., 1996) we set up a strategy to identify RA/RX downstream genes of mouse and human development that are possible candidate genes involved in the generation of congenital malformations. By RACE-PCR of trapped ES clones we identified a novel gene that is expressed in embryonic stages of mouse (8 d p.c. >>) and in different tissues of adult mouse and man. In northern analyses a cDNA fragment of the gene identifies a 2.5 kb transcript in mouse tissues and two transcripts of 2.7 kb and 3.0 kb in tissues of adult humans. By screening of mouse and human Marathon-cDNA libraries and analyzing homologous ESTs we identified the corresponding cDNAs including the various 3'UTRs. The mouse and human sequences of the gene share an identical ORF of 1512 bp and are homologous in 90 % of their nucleotide sequence and in 94 % of their amino acid sequence. Using mouse P1 and human BAC clones we characterized the genomic structure of the gene. Transcription factor scanning identified RA-responsive and RA-related orphan receptor-binding elements in the promoter region. The FISH analysis based on the human BAC located the gene within the chromosomal region 3q24-26. We expect, that the detailed analysis of the promoter region will unravel the retinoic acid responsive control mechanism for the gene and that a refined chromosomal mapping will help to correlate the identified gene with disorders mapped to the chromosomal region 3q24-26. (Supported by DAAD and DFG).

**The Mouse Homolog of Pyrin, the FMF Protein, Regulates Cytokine Production and Apoptosis.** *J.J. Chae<sup>1</sup>, H. Komarow<sup>1</sup>, G. Wood<sup>1</sup>, P.P. Liu<sup>2</sup>, D.L. Kastner<sup>1</sup>*. 1) Inflammatory Biology Section, Rheumatic Disease Genetics and Genomics Branch, NIAMS; 2) Oncogenesis and Development Section, Genetics and Molecular Biology Branch, NHGRI, NIH, Bethesda, MD.

We recently identified the gene for familial Mediterranean fever (FMF), a recessive disorder of inflammation. This gene, denoted *MEFV*, is highly expressed in granulocytes and activated monocytes, but the function of its protein product, pyrin, is unknown. To investigate pyrin's function, we cloned *Mefv*, the mouse homolog of *MEFV*, and generated *Mefv* knockout (KO) mice. *Mefv* transcripts are detected primarily in peripheral blood granulocytes and monocytes, and the predicted protein shares most, but not all, of the motifs found in human pyrin. Using an antibody to N-terminal mouse pyrin, we analyzed protein from in vitro-stimulated peritoneal monocytes. In wild-type mice, pyrin was highly induced by the anti-inflammatory cytokines IL-4 and IL-10, by proinflammatory agents lipopolysaccharide (LPS) and TNF- $\alpha$  at lower levels, and at highest levels by IL-4 and LPS together. Pyrin expression was not induced by TNF- $\alpha$  in TNFR1-KO mice, by IL-4 or IL-10 in JAK3-KO mice, or by any stimuli in NF- $\kappa$ B-KO or LPS-desensitized C3H/HeNHsd mice. Transient transfection of *Mefv* into the mouse monocytic cell line RAW264.7, which does not ordinarily produce pyrin, led to DNA fragmentation characteristic of apoptosis. When treated with LPS/IL-4, transfected cells produced substantially less IL-1 transcript and protein relative to nontransfected controls. We also examined the induction of apoptosis by IL-4/LPS in peritoneal monocytes from *Mefv* C-terminal KO mice, and found that cells from homozygous KO mice exhibited reduced DNA fragmentation relative to wild-type controls. These results suggest that pyrin acts as an anti-inflammatory molecule by inhibiting IL-1 synthesis and inducing monocyte apoptosis.

**Genotypic diagnosis of T<sup>B</sup><sup>+</sup> SCID by functional assay.** A.P. Hsu<sup>1</sup>, S.M. Anderson<sup>1</sup>, R. Buckley<sup>2</sup>, F. Candotti<sup>1</sup>, G. Uzel<sup>1</sup>, J.M. Puck<sup>1</sup>. 1) NHGRI/GMIBB, NIH, Bethesda, MD; 2) Duke Univ Med Center, Durham, NC.

Severe combined immune deficiency (SCID) is a group of disorders affecting both cellular and humoral immunity. Affected patients die of recurrent infections unless immunity is restored by bone marrow transplant or gene therapy. Most SCID cases are T<sup>B</sup><sup>+</sup>, predominantly due to X-linked mutations in *IL2RG*. However autosomal recessive phenocopies include Janus kinase 3 (*JAK3*) and Interleukin 7 receptor  $\alpha$  (*IL7RA*) deficiency. Determination of the genetic defect is crucial for accurate genetic counseling, carrier and prenatal diagnosis and gene therapy. *IL2RG* encodes the common gamma chain (gc) of cytokine receptor complexes for IL2, 4, 7, 9 and 15. Signaling is through heteromeric receptors to JAK proteins and signal transducers and activators of transcription (STATs). Determining the genetic basis of T<sup>B</sup><sup>+</sup> SCID involves ruling out mutations in *IL2RG* and performing laborious IP/western blots for JAK3 and IL7Ra. We have developed a flow cytometric assay to distinguish among *IL2RG*, *JAK3* and *IL7RA* mutations. IL2 or IL7 activate the gc/JAK3/STAT5 pathway in lymphocytes resulting in phosphorylated STAT5 (pSTAT5). In control B-cell lines fluorescence index (FI), ratio of geometric mean of pSTAT fluorescence in stimulated vs unstimulated samples, increases two-fold after IL2 or IL7 stimulation. Cells with gc or JAK3 defects fail to phosphorylate STAT5 resulting in a FI of 1.0. IL4 utilizes gc in its receptor but signals through JAK1 to STAT6. After IL4 stimulation, control cells have a FI near 3.0. *IL2RG* deficient cells, lacking a complete receptor, have a FI of 1.0. *JAK3* deficient cells have an intermediate FI of 1.6. Comparison of the FI of STAT5 and STAT6 after IL2, IL4 or IL7 stimulation makes possible the identification of specific blocks in activation due to mutations in gc, JAK3 or IL7Ra. Targeted sequencing of the appropriate gene then leads to mutation detection. Genotype/phenotype correlation and functional consequences of amino acid substitutions can be evaluated utilizing this signal transduction assay. Appropriate counseling and testing for relatives of T<sup>B</sup><sup>+</sup> SCID patients can be provided and gene therapy for patients can be considered.



**Histone Acetylation and Methylation Patterns of the Human Imprinted *NDN* Gene.** *J.C.Y. Lau, M.L. Hanel, R. Wevrick.* Dept Medical Genetics, Univ Alberta, Edmonton, AB., Alberta, Canada.

A subset of mammalian genes display imprinted expression where monoallelic silencing is observed that is dependent on the parent of origin of the alleles. This differential marking of the parental genomes occurs in gametogenesis, and is switched when passing through the germ line of the opposite gender. The allele-specific expression is initiated by an unknown epigenetic mark and can be propagated through development to direct expression in adult tissues. The mechanism by which imprinted genes are marked has not been fully elucidated. Evidence suggests involvement of allele specific DNA methylation and differential histone acetylation. The imprinted domain on 15q11-q13 contains a cluster of co-ordinately regulated maternally and paternally expressed genes, as well as several biallelically expressed genes. Some of these genes have been implicated in the human genetic disorders Prader-Willi Syndrome (PWS) and Angelman Syndrome (AS). The imprinted genes in the region are under control of a cis acting Imprinting Centre (IC) located at the 5' end of the *SNRPN* gene. The IC has been reported to be associated with allele specific DNA methylation and local histone acetylation differences. As these differences can have significance in *SNRPN* regulation and/or IC function, we studied an imprinted gene not co-localizing with the IC. To this end, we have characterized DNA methylation and histone acetylation patterns in the *NDN* gene, encoding neudin, an imprinted target ~1 Mb proximal to the IC. Differential methylation and histone acetylation was found to be associated with *NDN*. Comparison of methylation states in expressing and non-expressing tissues indicate DNA hypomethylation is associated with the paternal allele regardless of expression in different cell types. Hyperacetylation in expressing cells have correlated with hypomethylation. Our results suggest DNA methylation and histone acetylation may mark paternal and maternal alleles differentially even in the absence of transcription. The IC may therefore direct allelic transcriptional potential and other cell specific factors are required for transcription.

**Identification and characterization of an antisense transcript to the *MEST* gene on 7q32 as a candidate imprinted**

**locus.** *K. Nakabayashi*<sup>1</sup>, *K. Mitsuya*<sup>2</sup>, *M. Meguro*<sup>2</sup>, *M. Oshimura*<sup>2</sup>, *S.W. Scherer*<sup>1</sup>. 1) Dept Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept. of Molecular and Cell Genetics, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan.

Genomic imprinting is an unusual mode of gene regulation causing the nonequivalent representation of the maternal and paternal genomes. This phenomena can influence mammalian development, growth, and behavior. Imprinted gene(s) on human chromosome 7 are thought to be involved in Russell-Silver syndrome (RSS) based on the fact that 10% of patients shows maternal uniparental disomy of chromosome 7. Although chromosome 7 contains three known imprinting loci, *GRB10* on 7p12, *PEG10* on 7q21 and *MEST* at 7q32, an involvement of the known imprinted genes in RSS has yet to be established. To screen systematically for novel imprinted genes, we have established somatic hybrid cell lines containing a paternal or maternal human chromosome 7 (in which the imprinting of *MEST* is maintained). Because imprinted genes are often clustered, thirty transcripts located between D7S530 and D7S649 (an interval of approximately 1.0 Mb encompassing *MEST*) were subjected to RT-PCR analysis using the somatic cell hybrids. Several transcripts showed allelic expression bias. These transcripts are being subjected to allelic expression analysis using human tissue material to evaluate the imprinting status in vivo. One transcript reproducibly showed paternal-specific expression in the somatic cell hybrid assay. This transcript is located in an intron of one isoform of *MEST* but is transcribed in the opposite direction to the gene. Based on these features, we tentatively named this antisense transcript *MEST-AS*. Northern blot analysis detected a 3.7 kb signal in RNA from paternal but not maternal hybrids. The expression of *MEST-AS* was detected in brain and testis among 12 human tissues tested by RT-PCR. Analysis of the mRNA sequence also revealed (i) the transcript is composed of at least two exons and (ii) that it does not contain any significant open reading frame suggesting the transcript may not encode a protein. We hypothesize *MEST-AS* may be involved in tissue-specific regulation of *MEST* gene.

***In vivo* DNaseI sensitivity studies reveal parent-of-origin-dependent differential chromatin structure in a 47 kb region containing the *Snurf-Snrpn* locus.** *J.R. Khadake*<sup>1</sup>, *R.D. Nicholls*<sup>2</sup>, *D.J. Driscoll*<sup>3</sup>, *T.P. Yang*<sup>1</sup>. 1) Department of Biochem. & Mol. Biol., Univ. Florida, Gainesville, FL; 2) Dept. of Psychiatry, Univ. Pennsylvania, Philadelphia, PA; 3) Dept. of Pediatric Genet., Univ. Florida, Gainesville, FL.

Mouse chr. 7C contains a cluster of imprinted genes spanning a region of 2 Mb which, is orthologous with the imprinted human Prader-Willi/Angelman syndrome (PWS/AS) region on chr. 15q11-q13. Microdeletions in PWS patients and deletion studies in mice have revealed the importance of the 5' region of the *SNURF-SNRPN* gene in regulating imprinted gene expression across these imprinted domains. PWS deletions in this region are associated with the loss of paternal gene expression and differential methylation of the entire imprinted gene cluster. To understand these long-range effects and to map potential *cis*-acting regulatory elements involved, we have analyzed the chromatin structure of 47 kb of the *Snurf-Snrpn* locus in mouse brain cells for DNaseI hypersensitive sites on the paternally and maternally-inherited alleles. Allele specificity of the hypersensitive sites was studied using transgenic PWS and AS insertion-deletion mice that carried either the maternally or paternally-inherited genes, respectively. We found that the major transcription initiation site of *Snurf-Snrpn* is flanked on either sides by hypersensitive sites that are most prominent on the paternal allele. Our studies also identified sites of weaker hypersensitivity that are present on both the maternal and paternal alleles. Weak hypersensitive sites were detected on the maternal allele upstream and downstream of the hypersensitive sites in the promoter region. Accessibility of the maternal and paternal alleles to DNaseI is also being studied to determine the state of chromatin in this region. The differential parent-of-origin-specific chromatin structure revealed by DNaseI hypersensitivity is likely to reflect regions that interact with *trans*-acting factors. These regions are likely to be involved in regulation of *Snurf-Snrpn* gene transcription and/or play a role in control of imprinted gene expression from the paternal mouse chr. 7C region.

**Cloning and characterization of novel members of the TRP superfamily in man and mouse: the MCOLN gene family.** *J.L. Falardeau*<sup>1,2</sup>, *J.C. Kennedy*<sup>1,2</sup>, *J.S. Acierno jr*<sup>1,2</sup>, *S.A. Slaugenhaupt*<sup>1,2</sup>. 1) Molecular Neurogenetics Unit, Massachusetts General Hospital, Boston, MA; 2) Harvard Institute of Human Genetics, Boston, MA.

Mucopolipidosis type IV (MLIV) is an autosomal recessive lysosomal storage disorder characterized by neurologic and ophthalmologic abnormalities. The gene responsible for this disorder, *MCOLN1*, has been cloned and shown to be defective in all MLIV patients. We have cloned the mouse homologue, *Mcoln1*, which shows 90% amino acid and 85% nucleotide identity to *MCOLN1*. *Mcoln1* maps to mouse chromosome 8 and contains an open reading frame of 580 amino acids. The mouse *Mcoln1* gene encodes two transcripts of 2.4 and 4.4 kb, while a single 2.4 kb transcript is seen in humans. Characterization of the larger *Mcoln1* transcript led to the discovery of a novel splice variant that encodes a 611 aa protein that differs in the c-terminal cytoplasmic tail. The identification of the mouse homologue is crucial to the development of mouse models for MLIV, and further investigation of the disorder.

Two additional members of the MCOLN gene family have been identified along with their mouse homologues. *MCOLN2* and *MCOLN3* both show a high degree of similarity to *MCOLN1*. Structural analysis of the amino acid sequences of the MCOLN gene family members predicts a protein motif consistent with the Transient Receptor Potential (TRP) family of proteins. All TRPs contain 6 transmembrane domains and are putative cation channels. The highest degree of synteny among the MCOLN family members occurs at the region of the 3rd through 6th transmembrane domains, including the putative cation pore. *MCOLN1* has been classified into its own TRP subfamily, TRPML. We propose that *MCOLN2* and *MCOLN3* are also members of the TRPML family. The identification of mutations in *MCOLN1* represents the first example of a neurological disease caused by a TRP-related channel.

**Complex organization and transcription profiles in the hyaluronidase gene region of mouse chromosome 9.** *T.L. Shuttleworth*<sup>1</sup>, *B.A. Wicklow*<sup>1</sup>, *M.D. Wilson*<sup>2</sup>, *B.L. Triggs-Raine*<sup>1</sup>. 1) Dept. of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, Manitoba, Canada; 2) Dept. of Biology, University of Victoria, Victoria, British Columbia, Canada.

Hyaluronidases are required for the breakdown of hyaluronan, an extracellular matrix glycosaminoglycan that is important in many cellular processes involving cellular proliferation and differentiation. Our laboratory has shown that mutations in the gene encoding human hyaluronidase 1, *HYAL1*, result in a mild lysosomal storage disorder, Mucopolysaccharidosis IX. The mild nature of the disorder led to the recognition of a family of three hyaluronidases located in tandem on human chromosome 3p21.3. We characterized a corresponding region of about 18 Kb on mouse chromosome 9 that contained, in order, *Hyal2*, *Hyal1*, and *Hyal3*. *Hyal1* and *Hyal3* were shown by molecular approaches to contain four exons and three introns. Another gene, *Fus2*, encoding an N-acetyltransferase, was partially located within intron 1 of *Hyal3*. Bioinformatic analyses and comparison of the human and mouse genomic sequences in this region confirmed the organization of the mouse genes and suggested a slightly different genomic organization in humans, with the entire *FUS2* gene located within the first intron of *HYAL3*. Northern blot analysis of adult mouse tissues revealed tissue expression profiles for *Hyal1* and *Hyal3* similar to their human counterparts but with more complexity in the number of transcripts representing the genes. Some larger transcripts were shown by Northern blot and RT-PCR to result from the co-transcription of *Hyal1* and *Fus2*; similar analyses demonstrated a cotranscript containing *Hyal1* and *Hyal3*. Our Northern analyses also showed that *Fus2*, whose expression is limited largely to the testis in humans, has acquired an expression profile similar to *Hyal1* in mouse. Transcripts containing *Hyal1* and *Fus2* are abundant, and have the potential to encode novel proteins. Further studies are needed to determine if the larger transcripts containing *Hyal1* and *Fus2* are translatable. This will allow the design of appropriate targeting vectors for mouse knockout models. Funded by CIHR (BTR) and an MHRC studentship (TLS).

**Functional Human Arylsulfatase A Expression in Insect cells.** *Y. Wang, M. Wang, X. Qi and Human Genetics.*  
Human Genetics, Children's Hospital , Cincinnati, OH.

Arylsulfatase A (ASA) is a lysosomal enzyme that catalyzes the hydrolysis of cerebroside sulfate and a variety of sulfate esters. Deficiency of ASA causes metachromatic leukodystrophy (MLD) through the abnormal accumulation of sulfatide. To study the structure-function relationship in ASA we expressed the recombinant human arylsulfatase A (rhASA) in Sf9 insect cells using a baculovirus system with full length hASA cDNA cloned into Bac-N-Blue vector. A high level of rhASA was secreted into the medium with the yield of rhASA in the serum-free medium up to 0.2 mg/ml. The culture medium was collected after the Sf9 insect cells were transfected (concentration:  $1-2.5 \times 10^6$  cells/ml and MOI: 15-35 virus/cell) by recombinant virus stock solution (activity  $8.9 \times 10^9$  pfu/ml). rhASA activity was assayed using artificial chromogenic substrate 4-nitrocatechol sulfate. The specific activity of the rhASA was 85% of native ASA in human brain fibroblast cells as evaluated by the cross-reacting immunologic material (CRIM)-specific activity assay. The expressed rhASA was purified and displayed a single band on an SDS-PAGE gel. Western blot analysis showed a 62 KDa rhASA molecule using rabbit anti-human ASA polyclonal antibody . This antibody had no cross-reactivity with arylsulfatases B and C. An enzymatic deglycosylation assay indicated that the purified rhASA was slightly glycosylated. Additionally, an MS based ASA activity assay was established using the native substrate, palmitoyl sulfatide. ASA activity stimulated by saposin B was measured with a detergent-free system. A number of rhASA mutant proteins were generated by site-directed mutagenesis to study post translational modification and metal binding functions. The present study provides a useful system for investigating the function - structure relationship of human ASA. .

**Impaired conditioned fear and enhanced long-term potentiation in Fmr2 knockout mice.** *Y. Gu<sup>1</sup>, K.L. McILwain<sup>1</sup>, E.J. Weeber<sup>2</sup>, T. Yamagata<sup>1</sup>, B. Xu<sup>1</sup>, B.A. Antalffy<sup>3</sup>, D. Armstrong<sup>3</sup>, C. Reye<sup>3</sup>, H. Zoghbi<sup>1,4,5</sup>, J.D. Sweatt<sup>2</sup>, R.E. Paylor<sup>1,2</sup>, D.L. Nelson<sup>1</sup>.* 1) Molecular and human genetics, Baylor College of Medicine, Houston, TX; 2) Division of neuroscience, Baylor College of Medicine; 3) Department of Pathology, Baylor College of Medicine; 4) Department of Pediatrics, Baylor College of Medicine; 5) Howard Hughes Medical Institute.

FRAAXE mental retardation (MR) results from expansion and methylation of a CCG trinucleotide repeat located in exon 1 of the X-linked FMR2 gene, which results in transcriptional silencing. The product of FMR2 is a member of a family of proteins rich in serine and proline, members of which have been associated with transcriptional activation. We have developed a murine Fmr2 gene knockout model by replacing a fragment containing parts of exon 1 and intron 1 with the *E. coli* lacZ gene, placing lacZ under control of the Fmr2 promoter. Expression of lacZ in the knockout animals indicates that Fmr2 is expressed in several tissues, including brain, bone, cartilage, hair follicles, lung, tongue, tendons, salivary glands, and major blood vessels. In the central nervous system, Fmr2 expression begins at the time that cells in the neuroepithelium differentiate into neuroblasts. Mice lacking Fmr2 showed impairment of both contextual and cued conditioning in the conditioned fear paradigm. Long-term potentiation (LTP) was found to be enhanced in hippocampal slices of Fmr2 KO compared to WT littermates. To our knowledge, this mouse knockout is the first example of an animal model of human mental retardation with impaired learning and memory performance and increased LTP. Thus, while a number of studies have suggested that diminished LTP is associated with memory impairment, our data suggest that increased LTP may be a mechanism that leads to impaired cognitive processing as well.

***MECP2* mutations in Rett syndrome negatively affect lymphocyte growth but do not affect imprinted expression in blood or brain.** *D. Balmer*<sup>1</sup>, *R.C. Samaco*<sup>1</sup>, *H.Y. Zoghbi*<sup>2</sup>, *J.M. LaSalle*<sup>1</sup>. 1) Med Micro & Immuno, UC Davis Sch Med, Davis, CA; 2) Med & Hum Genet & HHMI, Baylor Col Med, Houston, TX.

Rett Syndrome (RTT) is an X-linked dominant neurodevelopmental disorder caused by mutations in *MECP2*, encoding methyl-CpG-binding protein 2 (MeCP2). Due to random X-inactivation, female somatic cells are mosaic for expression of mutant *MECP2*. We therefore performed single cell cloning of T lymphocytes from four RTT patients with known *MECP2* mutations to isolate cells expressing mutant or wild-type *MECP2*. The frequency of *MECP2* mutant-expressing clones was compared to the percentage of mutant allele expression by RT-PCR on RNA derived from bulk lymphocytes. Mutant-expressing clones were present at a significantly lower frequency ( $P < 0.0001$ ) than expected. These results demonstrate that although *MECP2* is not essential for lymphocyte growth, expression of the *MECP2* mutation causes a growth disadvantage *in vitro*. Since MeCP2 is thought to silence transcription of methylated genes by recruiting Sin3A and histone deacetylase (HDAC), we hypothesized that MeCP2 may be required for silencing imprinted or methylated gene expression. The allelic expression of three different imprinted genes (*SNRPN*, *IPW* and *IGF2*) was examined by RT-PCR and RFLP analysis and demonstrated normal monoallelic expression of all RTT clones. To test the possibility that MeCP2 could regulate imprinted gene expression exclusively in brain, we examined the expression of 5 imprinted genes (*SNRPN*, *IPW*, *NECDIN*, *H19* and *IGF2*) in 4 RTT brain samples and also observed monoallelic expression. In addition, RTT lymphocyte clones were tested for expression of *IFNG*, a nonimprinted but methylated gene differentially expressed in T cell clones. *IFNG*-expressing and nonexpressing clones were observed independent of *MECP2* allelic expression, suggesting that *MECP2* mutations do not reverse methylation-induced silencing of this tissue-specific gene. Although the HDAC inhibitor Trichostatin A did not alter *SNRPN* expression, *IFNG* was induced in a previously *IFNG*-nonexpressing *MECP2*-mutant-expressing clone. In conclusion, our results do not support an essential role for either MeCP2 or HDAC in the silencing of imprinted genes.



**Identification of altered gene expressions on specific chromosomes in ICF syndrome cells using oligonucleotide microarrays.** *P. Jin, M.D. Kaytor, S.T. Warren.* Howard Hughes Medical Institute and Department of Genetics, Emory University School of Medicine, Atlanta, GA.

ICF syndrome is a rare autosomal recessive disease characterized by immunodeficiency, centromeric decondensation and facial anomalies, caused by mutations in the putative *de novo* DNA methyltransferase 3B (DNMT3B) gene. In ICF syndrome, the classical satellite II and III regions of chromosomes 1, 9 and 16 are hypomethylated, which leads to chromosomal instability. Despite these cytogenetic abnormalities, how the mutations in DNMT3B affect gene expression remains unclear. Here, using oligonucleotide microarrays we investigated changes in gene expression in ICF syndrome cells. Two B cell lines from ICF syndrome patients and three controls were used. Of 12,000 genes interrogated, over one hundred genes showed consistent changes in the two ICF cells compared to all control cells. Interestingly, one third of these genes are located on chromosome 1 and 6, but not on chromosome 9 and 16. Among the genes altered in ICF cells, several genes including SCHIP-1, RBPMS, PTPRK and UBD, are expressed only in ICF cells but not in any control cell. Since ICF syndrome is caused by the defects in DNA methylation, it is likely that the mutation in DNMT3B leads to demethylation and subsequent activation of these genes in ICF cells, which are transcriptionally repressed in control cells. The methylation status of these genes promoters is being evaluated. These studies will help us understand not only the molecular basis of ICF syndrome but also the roles of DNA methylation in human diseases.

**A canine model for Lafora's progressive myoclonus epilepsy.** *B.A. Minassian<sup>1</sup>, S.N. Fitzmaurice<sup>2</sup>, C. Rusbridge<sup>3</sup>, R.J.M. Franklin<sup>4</sup>, E. Young<sup>1</sup>, L. Ianzano<sup>1</sup>, G.D. Shelton<sup>5</sup>, S.W. Scherer<sup>1</sup>.* 1) Hosp Sick Children, Toronto, Canada; 2) Wey Referrals, Woking, UK; 3) Atkinson Morley's Hosp, Wimbledon, UK; 4) U of Cambridge, UK; 5) U of California, San Diego.

Lafora's disease (LD) is a severe autosomal recessive progressive myoclonus epilepsy. Myoclonic and photically induced seizures begin between ages 6 and 15 years in previously normal children and continue until death. Pathognomonic accumulations of an abnormal glucose polymer (polyglucosans; Lafora bodies) are found in most tissues including the perikarya of neurons. Mutations in at least two genes cause LD. One gene, EPM2A, encodes a rough endoplasmic reticulum-associated dual-specificity phosphatase. The locus for the other, much rarer, form(s) is unknown. LD has been documented in autopsies of several animals including parakeets, foxes, cats, dogs and cows. However, there is presently no natural or genetically engineered animal model for the disease. Because of the relatively frequent reporting of LD in animals, we sought to identify a natural animal model. We studied several dog breeds with autosomal recessive epilepsies. In the miniature wirehaired dachshunds (MWHD), affected individuals exhibited myoclonic and photoconvulsive seizures starting between ages 6 and 13 years and progressively worsening with time. Muscle biopsy showed polyglucosan accumulations, and brain at autopsy revealed typical Lafora bodies in neuronal perikarya. In order to determine the mutation in these animals, we screened a canine bacterial artificial chromosome library with each of the four exons of the human EPM2A gene and identified overlapping clones containing the corresponding dog *epm2a* exons. Sequencing of these exons and surrounding introns is in progress (90% complete). So far we have measured 95% identity between dog and human at the translated amino acid level. Interestingly, we also detected ~61% identity in the 5'UTR and intronic sequences (this was not observed in human and mouse comparisons). The affected MHWD belong to an extended pedigree with multiple affected members. This breed will serve as a model for the EPM2A form of LD, or will facilitate mapping of the other LD gene(s).

**mRNA may carry spatial genetic information-the topological configuration of mRNA affect the initial structure of nascent polypeptides.** *C.Q. Liu, S.Q. Liu.* Laboratory of cellular and molecular evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming YN, 650223, P. R. China.

The hypothesis of "Three-dimensional genetic information flow" put forward by us describes that mRNA's structure can affect protein's folding, it indicates mRNA carries three-dimensional genetic information. The nonuniform of translational rate exert a phenotypic effect on the folding of encoded protein. We explored the factors that can affect translational rate through modeling structure of single-stranded mRNA (PDB ID is 1ILI) and ribosomal model, Some significant information was obtained: i. After mRNA hairpin is unfolded, the conformation of the single stranded mRNA is close relative to that of the corresponding mRNA hairpin structure, and it is helical region alternating with extended or coiled region when it is read by ribosome, such conformation of single stranded mRNA affect the translational speed. ii. we constructed the models that showed how the tRNA-mRNA interacted and orientated on ribosome. iii. Based on the model of ribosomal functional center and the novel structural information we gained, a new ribosomal model was built. iv. From the model of interaction between mRNA and tRNA, we observed that through the anticodon of relative rigid tRNA pairing with the codon of mRNA the distance between mRNA and CCA end of tRNA keep relative constant, and the shape of the connection line of the CCA ends was analogous to that of mRNA, it suggest that there exists relationship between the conformation of mRNA and that of nascent polypeptide. Our results imply that mRNA not only carry one-dimensional linear genetic information, but also possibly carry three-dimensional genetic information. The conformation of single stranded mRNA unfolded from hairpin of template mRNA can affect translational rate and initial structure of nascent polypeptide. Such brand-new translation mechanism has important biological significance for understanding the process of genetic information transferring from mRNA to protein.

**Development and characterization of a conditional M6p/Igf2r knockout mouse.** *S.K. Murphy<sup>1</sup>, A.A. Wylie<sup>1</sup>, A.J. McVie-Wylie<sup>2</sup>, D. Pulford<sup>1</sup>, C.M. Nolan<sup>1</sup>, T.C. Orton<sup>3</sup>, R.L. Jirtle<sup>1</sup>.* 1) Radiation Oncology; 2) Medical Genetics, Duke University Medical Center, Durham, NC; 3) Molecular Toxicology, AstraZeneca, UK.

The mannose-6-phosphate/Insulin-like growth factor 2 receptor (M6p/Igf2r) encodes for a multifunctional protein involved in lysosomal enzyme trafficking, tumor suppression and T-cell mediated immunity. M6p/Igf2r is also imprinted in mice with exclusive expression from the maternal allele. Previous M6p/Igf2r knockout studies resulted in neonatal lethality thus preventing any further analysis on the multifunctional role of the M6p/Igf2r during adulthood. To help elucidate the role of this gene in adult mice, we have generated conditional M6p/Igf2r knockout mice using the cre/loxP system. A transgenic line was produced in which M6p/Igf2r exon 10 was flanked by two loxP sites.

Concomitant expression of cre recombinase in cells with a floxed M6p/Igf2r exon 10 resulted in the creation of a null allele through the excision of exon 10 sequence and the introduction of a premature stop codon in exon 11. Germline inheritance of a maternal allele lacking exon 10 sequence due to cre-mediated excision resulted in a neonatal lethal phenotype identical to that observed in the original knockout study. Subsequently, tissue-specific knockout mice were produced harboring a null maternal M6p/Igf2r allele in either the liver or cardiac/skeletal muscle. Both knockout lines of mice are viable and the absence of M6p/Igf2r in the tissues of interest was confirmed by western blot analysis. Comparison of either the liver-specific or muscle-specific M6p/Igf2r knockout animals with wild-type littermates at 3 months and 12 months of age revealed no significant phenotypic or histological differences. M6p/Igf2r expression does not therefore appear to be critical to the normal function and maintenance of adult liver or muscle, however, the mechanistic role M6p/Igf2r plays in response to cellular stress remains to be determined. The successful creation of viable, tissue specific, M6p/Igf2r knockout mouse models will now allow detailed analysis of M6p/Igf2r function in a number of cellular processes including carcinogenesis, lysosomal trafficking and T-cell mediated immunity.

**Targeted disruption of the Nijmegen breakage syndrome gene NBS1 leads to early embryonic lethality in mice.**

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Nijmegen breakage syndrome (NBS) is a rare human autosomal recessive disorder characterized by microcephaly, immunodeficiency, and a significantly increased incidence in cancer. Phenotypically, NBS cells display chromosome instability, radiation sensitivity, and impaired cell cycle checkpoint control in response to ionizing radiation. We and others identified NBS1, the gene mutated in NBS patients as well as Nbn, the murine homologue of NBS1. Approximately 90% of NBS patients are homozygous for the 657del5 mutation of NBS1 that causes premature termination at codon 219. Since it is unknown whether the 657del5 mutation in NBS patients is a hypomorphic or null mutation, it is possible that complete inactivation of NBS1 might reveal novel functions for this protein. We therefore targeted the 5' region of the Nbn gene to produce a null allele. From Nbn<sup>+/-</sup> targeted ES cell clones chimeric mice were generated that transmitted the germline Nbn mutation to offspring. Nbn<sup>+/-</sup> mice were viable and healthy and grew normally. Nbn<sup>+/-</sup> mice did not display immune defects, as measured by flow cytometric analysis using a variety of T cell (lineage) markers and B cell (lineage) markers. Nbn<sup>+/-</sup> mice were fertile, but when interbred, homozygous mutants for the targeted Nbn allele were not found among the 98 offspring ( $p < 0.0001$ ) analyzed from one founder line. Thus it appears that Nbn, and by extension NBS1 is essential for growth of early embryonic cells. We determined the onset of lethality at embryonic day 6.5 (e6.5) and found several resorbed embryos at e7.5-e9.5. The timing of this apparent developmental block in Nbn<sup>-/-</sup> embryos is similar to that for knockouts of Rad50 and Mre11, whose products complex with nibrin. The results suggest that the observed phenotypes in NBS patients may not result from a complete inactivation of NBS1 but rather from hypomorphic truncation mutations that are compatible with cell viability. To test this hypothesis we are using full length NBS1 and various mutant forms to rescue the embryonic lethality of the Nbn<sup>-/-</sup> mice.

**The MJD1 homologue in *C. elegans* is expressed ubiquitously and is essential for egg laying and hatching.** *C. Santos*<sup>1</sup>, *M. Ailion*<sup>4</sup>, *J. Thomas*<sup>4</sup>, *J. Sequeiros*<sup>1,2</sup>, *P. Maciel*<sup>1,2,3</sup>. 1) UnIGENE, IBMC, Univ Porto; 2) ICBAS, Univ Porto; 3) ISCS-Norte, Paredes, Portugal; 4) Dept Genetics, Univ Washington, Seattle.

Machado-Joseph disease (MJD) is a neurodegenerative disorder caused by the expansion of a CAG/polyglutamine repeat within the coding region of the MJD1 gene. The function of the MJD1 gene product, ataxin-3, remains unknown.

We have identified a *C. elegans* gene encoding a protein with a high degree of homology to human ataxin-3, presenting 38 % identity and 56% similarity at the aminoacid level, suggesting evolutionary conservation. The *C. elegans* gene consists of 4 exons, encoding a protein with 317 aminoacids, which does not contain a glutamine tract.

In order to study the expression pattern of this MJD1-like gene in *C. elegans* we constructed a fusion protein using the genomic sequence of the gene, including the endogenous promoter region, and a GFP (green fluorescent protein) expression vector. The worm line obtained after injection of this construct showed a strong fluorescence during all stages of postnatal development. In the adult hermaphrodite, expression could be seen in many tissues like hypodermis, gut, neurons in the head and tail, spermatheca and coelomocytes.

Silencing of the gene expression using bacterial-mediated RNAi, originated morphological abnormalities in the adult hermaphrodite, with swelling and distortion of internal body structure due to accumulation of eggs. These were arranged in a disorganized manner, quite different from the regular disposition in the wild-type animal. Movement of the worms was slightly slower than usual. A small percentage of the animals showed protrusion of the vulva, and in another subgroup the eggs hatched inside the mother ("bag-of-worms" phenotype), leading to death of the animal.

Further analysis is needed to clarify in more detail the role of this gene in development and to confirm that it is the worm orthologue of the MJD1 gene.

**Interaction analyses elucidate the pathophysiology of HCM-causing missense mutations in cardiac myosin binding protein-C.** *J. Moolman-Smook<sup>1</sup>, L. Korkie<sup>1</sup>, T. DeLange<sup>1</sup>, C. Redwood<sup>2</sup>, H. Watkins<sup>2</sup>, V. Corfield<sup>1</sup>.* 1) US/MRC Centre for Molecular and Cellular Biology, University of Stellenbosch Health Sciences Faculty, SA; 2) Dept Cardiovascular Medicine, University of Oxford, UK.

Despite its identification as an integral thick filament protein ~30 years ago, the function of cardiac myosin binding protein-C (cMyBPC) has only become clear recently, while its sarcomeric arrangement is still speculative. Yet, understanding both facets is crucial due to its role in hypertrophic cardiomyopathy (HCM), a common genetic cardiomyopathy. The pathophysiology of HCM-causing truncation mutations in cMyBPC relate to loss of the thick filament-binding sites; however, the pathophysiology of missense mutations in domains of the protein with as-yet undefined interactions is unknown. C5 is one such domain, in which two HCM-causing missense mutations have been described.

We used the yeast two-hybrid system to screen a cardiac cDNA library for the ligand of domain C5, and investigated the effect of the HCM-causing R654H and N755K mutations on this interaction with quantitative B-galactosidase assays.

When more than  $7 \times 10^6$  clones were screened, domain C5 preferentially bound to clones encoding other domains of cMyBPC. The interaction was narrowed to domain C8 by deletion mapping. This interaction was significantly strengthened by the R654H mutation, and more so by the N755K mutation ( $p < 0.0001$ ).

Our data are consistent with a model for the sarcomeric arrangement of cMyBPC in which consecutive cMyBPC molecules trimerize into a tight collar around the thick filament, with overlaps of domains C5-C7 of one cMyBPC with domains C8-C10 of the next, with conservation of protein orientation. This interaction is likely to be dynamically formed and released during the phosphorylation cycle of cMyBPC, a process which is hindered by the tighter bonds introduced by the R654H and N755K mutations. This model synthesizes data from previous biochemical and structural studies, and for the first time, sheds light on the pathophysiology of HCM-causing missense mutations in cMyBPC.

**Ataxin-1 Binds a Brain-Specific RNA.** *H.G. Serra<sup>1</sup>, L.A. Duwick<sup>1</sup>, C.E. Byam<sup>1</sup>, H.Y. Zoghbi<sup>2</sup>, H.T. Orr<sup>1</sup>.* 1) Lab Medicine & Pathology, Univ Minnesota, Minneapolis, MN; 2) H. Hughes Medical Institute, Baylor College of Medicine, Houston, TX.

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disease caused by the expansion of a polyglutamine repeat within ataxin-1. Recently, we demonstrated that ataxin-1 binds to RNA in vitro with decreasing affinity as the length of the polyglutamine repeat increases. As an approach to isolate and characterize in vivo RNA targets of ataxin-1, we synthesized biotinylated ataxin-1 that was further coupled to avidin resin making an affinity column. Nuclear extract from FVB mouse cerebellum was incubated with the affinity resin and the bound RNA population was isolated. The subpopulation of RNA specifically bound to ataxin-1 was then amplified by differential display RT-PCR and analyzed on a denaturing gel. Bands specifically bound by ataxin-1 were excised from the gel, reamplified, cloned and sequenced. We isolated a 7kb specific ataxin-1 bound mRNA (ABR-14) that is expressed only in brain and shows differential levels of expression among our different transgenic mouse lines. ABR-14 is downregulated in the affected ataxin-1 mutant mice that carry an SCA-1 allele with 82 CAG repeats (BO5). The gene is also downregulated in ataxin-1 knockout mice (SCA1<sup>-/-</sup>). In addition, this mRNA is upregulated in two other non-ataxic SCA-1 transgenic mouse lines. One of these lines expresses a wild type SCA-1 allele with 30 CAG repeats (AO2), the other contains 82 repeats and only expresses the SCA1 gene in the cytoplasm due to a point mutation in the nuclear localization signal (K772T). ABR-14 is presently being sequenced to determine the protein encoded by this mRNA. Subsequent studies will examine its role in SCA1.



**Transfection of MEFV, the gene causing Familial Mediterranean Fever, induces alterations in arachidonate lipoxygenation.** *Y. Torosyan, G. Wood, N. Raben, E. Mansfield, D.L. Kastner.* NIAMS/NIH, Bethesda, MD.

Familial Mediterranean fever (FMF) is an autoinflammatory disorder characterized by self-limited recurrent episodes of fever and aseptic leukocyte-mediated polyserositis. The causative gene, MEFV, was recently identified by positional cloning, and is expressed predominantly in granulocytes and activated monocytes. In the present study we examined the relationship between MEFV expression and the expression of two enzymes in the arachidonate pathway, 5-lipoxygenase (5-LOX) and 15-lipoxygenase (15-LOX). 5-LOX expression is restricted to myeloid cells and leads to the production of proinflammatory leukotrienes, while the more widely expressed, reciprocally regulated 15-LOX synthesizes antiinflammatory lipoxins. Since leukotriene levels are increased in the serum of FMF patients, we utilized multiplex RT-PCR to analyze 5- and 15-LOX gene expression as a possible function of MEFV expression. MEFV and 5-LOX demonstrated concordant expression profiles in monocytic cell lines: low levels in immature U937 cells and high levels in the more mature THP-1 cell line. HeLa cells (a fibroblast-like cervical carcinoma line) demonstrated low endogenous levels of MEFV and 5-LOX, which were upregulated by IL-4 (but not IL-13). Transient transfection of HeLa cells with wild-type MEFV upregulated 5-LOX expression to levels characteristic of leukocytes, and this effect was blocked by colchicine, the drug of choice in FMF. In addition, MEFV transfection altered the response of HeLa cells to cytokines. In transfected, but not untransfected, HeLa cells, both IL-4 and IL-13 induced 15-LOX expression, as is characteristic of monocytic cells. Interferon-gamma induced 15-LOX expression in untransfected, but not transfected, cells. MEFV transfection led to altered LOX expression patterns and cell survival (based on apoptotic rates by APO/BRDU) in HeLa cells in response to the proapoptotic agents CD95, staurosporine, NDGA, and colchicine. Thus, MEFV expression leads to changes in lipoxygenase gene expression, which probably play a role in both inflammation and apoptosis. cDNA microarray experiments are in progress to delineate more global effects of MEFV on gene expression profiles. *MEFV*.

**A novel FLNC interacting protein.** *I. Dalkilic*<sup>1</sup>, *T.G. Thompson*<sup>1</sup>, *M.A. Brosius*<sup>1</sup>, *A.A. Puca*<sup>1</sup>, *F. Muntoni*<sup>2</sup>, *L.M. Kunkel*<sup>1</sup>. 1) Department of Genetics, Children's Hospital, Boston, MA; 2) Neuromuscular Unit, Division of Pediatrics, Obstetrics, and Gynaecology, Imperial College School of Medicine, Hammersmith Hospital, London.

Mutations in genes encoding for the sarcoglycans (a-, b-, d-, g-) produce a limb-girdle muscular dystrophy phenotype; however, the precise role of this group of proteins in skeletal muscle is not known. We have recently identified a skeletal muscle specific form of filamin (FLNC or g-filamin) as a d- and g-sarcoglycan interacting protein. We have previously shown that FLNC localizes to both the sarcolemmal membrane and the Z-line in the contractile apparatus. Interestingly, in patients with sarcoglycanopathies or in mdx mice the percentage of FLNC in the membrane is greatly increased.

In order to better understand the role of FLNC in skeletal muscle we have also performed a yeast two-hybrid assay in the hope of finding novel interacting partners. One of the clones we isolated is a novel gene whose homolog in *C.elegans* gives an uncoordinated phenotype when mutated. This new gene maps to chromosome 1. It has been recently reported that two families with congenital muscular dystrophy (CMD1B) are genetically linked to a region in chromosome 1 containing this new clone. We have determined the genomic organization for this gene and currently analyzing DNA from these patients to determine if they have a disease causing mutation. We have also confirmed interaction of this protein with FLNC *in vitro* and are currently studying this interaction *in vivo*.

**Integrin is a compensatory transmembrane linkage to sarcoglycan in muscle.** *M. Allikian*<sup>1</sup>, *A.A. Hack*<sup>1</sup>, *S. Mewborn*<sup>1</sup>, *U. Meyer*<sup>2</sup>, *E.M. McNally*<sup>1</sup>. 1) Dept Medicine, University of Chicago, Chicago, IL., USA; 2) Wellcome Trust Centre for Cell-Matrix Research, Manchester UK.

Mutations in g-sarcoglycan cause autosomal recessive muscular dystrophy in humans and mice. In muscle, the sarcoglycan complex includes transmembrane proteins that normally associate with dystrophin and are secondarily reduced when dystrophin is mutated. Additionally, mutations in any single sarcoglycan gene produce instability of the entire sarcoglycan complex. Integrin  $\alpha 7$  is a transmembrane protein expressed mainly in skeletal and cardiac muscle that, like sarcoglycan, provides linkage between cytoskeletal elements and the extracellular matrix. Integrin  $\alpha 7$ , together with integrin  $\beta 1$ , constitute the major integrin type in mature skeletal muscle. Moreover, mice lacking integrin  $\alpha 7$  (Mayer et al. Nat. Gen. 1997) show muscle degeneration similar, but less severe, than those lacking g-sarcoglycan (Hack et al. J. Cell Biol. 1998). We found that integrin  $\alpha 7$  protein is upregulated at the plasma membrane in g-sarcoglycan null mice. To ascertain whether upregulation of integrin  $\alpha 7$  compensates for the loss of the transmembrane sarcoglycan linkage in sarcoglycan and dystrophin-deficient muscle, we bred mice lacking integrin  $\alpha 7$  with mice lacking g-sarcoglycan (*gxi*). These *gxi* mice exhibit profound rapid muscle degeneration leading to death at an average of 21-25 days of age. Histopathologic examination suggests pronounced and diffuse muscle degeneration as seen by Evans Blue dye uptake. Embryonic myosin heavy chain immunostaining of *gxi* muscle, together with normal in vitro fusion properties of *gxi* myoblasts indicates that *gxi* myotubes have normal regenerative capacity. Therefore, upregulation of integrin where sarcoglycan is absent reflects a compensatory transmembrane linkage for muscle cell attachment to the extracellular matrix. Furthermore, this argues that the function of sarcoglycan, like that of integrin, is as a mechanosignaling complex that integrates extracellular matrix signals for the growth and maintenance of myotubes.

**Gene expression analysis of muscle from Duchenne muscular dystrophy patients.** *J.N. Haslett, D. Sanoudou, S.A. Greenberg, H.G.W. Lidov, I.S. Kohane, A.H. Beggs, L.M. Kunkel.* Genetics Division, Children's Hospital, Harvard Medical School, Boston, MA.

Muscular dystrophy (MD) refers to a clinically and genetically heterogeneous group of myopathies defined by progressive degeneration of skeletal muscle fibers, leading to loss of muscle function. Mutations in the dystrophin gene were identified as causative of the most common muscular dystrophy, Duchenne MD. Dystrophin is linked to a group of integral membrane proteins, forming the dystrophin-associated protein complex (DAPC). To examine the DMD pathogenic pathways and identify new or modifying factors involved in muscular dystrophy we used Affymetrix oligonucleotide arrays to analyze the expression patterns of 12,600 genes in 12 DMD patients. The DMD data were compared to data from 10 normal skeletal muscle samples and to data obtained from other myopathic samples. Conventional statistical and fold-ratio comparisons and automated classification and clustering techniques were used to analyze the data, allowing identification of both disease-specific changes and changes due to the non-specific response of diseased muscle. This will enable additional analysis of DMD pathophysiological mechanisms and those of skeletal muscle more generally. When compared to other datasets, the DMD samples show a distinctive clustering pattern, presumed to reflect differences in the pathogenic pathways. Fold-ratio analysis suggests approximately 80 of the genes examined differ significantly in expression level between normal and DMD muscle. More genes were found to be overexpressed than underexpressed in DMD, potentially reflecting an increase in protein turnover, probably due to the degenerative and regenerative nature of the disease. As expected dystrophin was found to be substantially underexpressed in DMD muscle. In contrast, a number of other muscle genes (myosin, troponinT, alpha- and beta-tubulin, alpha-actinin) were found to be overexpressed in DMD muscle. The observed changes are being subjected to further statistical analysis and classification, in addition to experimental verification, to provide insight into the molecular mechanisms behind the DMD pathogenic pathways.

**Myne-1, a spectrin repeat protein of the myocyte inner nuclear membrane, interacts with A-type lamins.** *J.M.K. Mislow*<sup>1,2</sup>, *M.S. Kim*<sup>2</sup>, *E.M. McNally*<sup>2</sup>. 1) Department of Pathology, University of Chicago, Chicago, IL; 2) Department of Medicine, University of Chicago, Chicago, IL.

Mutations in the genes encoding the inner nuclear membrane proteins lamin A/C and emerin produce cardiomyopathy and muscular dystrophy in humans and mice. The mechanism by which these broadly expressed gene products result in tissue specific dysfunction is unknown. We have identified a protein of the inner nuclear membrane that is highly expressed in striated and smooth muscle. The primary sequence of myne-1 (myocyte nuclear envelope) predicts seven spectrin repeats, a serine-rich domain, and a single transmembrane domain at its carboxyl terminus. Between spectrin repeats five and six is an interrupted "LEM domain" so named for its presence in the nuclear envelope proteins MAN-1, emerin, and LAP2. The LEM domain of emerin has been shown to directly bind BAF, a protein shown to bridge and organize double-stranded DNA (dsDNA). Therefore myne-1 may play a role not only in structural integrity of the nuclear envelope through spectrin repeats, as spectrin does in erythrocyte membranes, but may also serve to localize dsDNA to the inner nuclear membrane. We found that myne-1 is expressed upon muscle differentiation in intranuclear foci concomitant with lamin A/C expression. In mature muscle, myne-1 and lamin A/C are perfectly colocalized, while colocalization with emerin is only partial. Moreover, we show that myne-1 and lamin A/C co-immunoprecipitate from differentiated muscle in vitro. Myne-1 maps to chromosomal 6q25, near the marker D6S420, placing it near two genetically mapped regions associated with cardiomyopathy. The muscle-specific inner nuclear envelope expression of myne-1, in conjunction with its interaction with lamin A/C, indicates this gene is a potential mediator of cardiomyopathy and muscular dystrophy.

**Homeobox Gene BP1 Expression Strongly Correlates with ER Expression in Human Breast Cancer.** *S. Fu<sup>1</sup>, A. Schwartz<sup>2</sup>, H. Stevenson<sup>1</sup>, G. Davenport<sup>1</sup>, P.E. Berg<sup>1</sup>.* 1) Dept Biochemistry & Molecular Biology, George Washington Univ Med Ctr, Washington, DC; 2) Dept Pathology, George Washington Univ Med Ctr, Washington, DC.

Homeobox genes are transcription factors that regulate a variety of genes involved in development and malignant transformation. In humans, we now report a newly identified divergent homeobox gene called Beta Protein 1 (BP1), functioning as a potential oncogene in human breast cancer. Previous studies in our laboratory demonstrated that BP1 is overexpressed in 47% of adult acute myeloid leukemia (AML) patients and 81% of pediatric AML patients, and that it is normally expressed in early hematopoietic progenitors. Overexpression of BP1 leads to increased growth and decreased erythroid differentiation in K562 leukemia cells, while reducing BP1 expression using antisense strategies leads to apoptosis. These data implicate BP1 as a survival factor and suggest a possible general mechanism by which BP1 could function as an oncogene. We demonstrated that BP1 is overexpressed in human breast cancer cell lines and tissues compared to normal epithelial cells and normal tissues by RT-PCR. Interestingly, BP1 overexpression strongly correlates with the tumorigenic potential in the cell lines examined. In tissues from 15 newly diagnosed breast cancer patients, we found that 73% expressed high levels of BP1 mRNA, whereas BP1 mRNA was undetectable in the remaining 27%. In contrast, BP1 was expressed (at a very low level) in only one of six normal breast tissues examined. BP1 expression was seen in 100% of the high grade, estrogen receptor (ER) negative, progesterone receptor (PR) negative tumor tissues and in 43% of ER positive, PR positive tumors. Additional breast tumors, as well as normal breast tissues, are being examined. These data suggest that BP1 may be a new marker in breast cancer for poor prognosis tumors and that it is a potential molecular target for therapy.

**Carboxylesterase 2 pharmacogenetics: intron/exon boundaries and polymorphism analysis.** *S. Marsh*<sup>1</sup>, *S.C. Pritchard*<sup>1</sup>, *C.M. Rose*<sup>1</sup>, *R. Donaldson*<sup>2</sup>, *P. Kwok*<sup>2</sup>, *H.L. McLeod*<sup>1,2,3</sup>. 1) Department of Medicine; 2) Department of Genetics; 3) Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St Louis, MO.

Carboxylesterase enzymes are found in several animal species and play an important role in drug metabolism and as a physiologic barrier to environmental toxins. There are carboxylesterases in a range of human tissues including placenta, brain and liver. Liver carboxylesterases 1 and 2 (CES1 and CES2) are involved in the conversion of the anticancer drug CPT-11 to its active form SN-38, a topoisomerase I poison. It has been recently shown that CES2 has a 12.5 fold higher affinity for CPT-11 compared to CES1. Differences in CES2 activity in patients receiving CPT-11 could have serious implications for drug efficacy and toxicity. The genomic structure of the CES1 gene has previously been characterized, with 14 exons and a gene size of 30kb. Currently little is known about the CES2 gene structure. Using *in silico* analysis and direct sequencing, CES2 was localized to 16q13-22.1 between markers D16S3031 and D16S3139, approximately 11.1cM telomeric from CES1. CES2 contains 12 exons ranging from 43 to 296bp and 11 introns ranging from 81 to 2335bp. The genomic sequence spans 1546bp, with an 8460bp cDNA sequence. CES2 shares 73% nucleotide homology with CES1. Variations in drug metabolizing enzymes can contribute to toxicity and/or altered sensitivity/resistance to drug treatment. SNP mining by *in silico* methods identified an a1242t polymorphism in exon 9 causing a Glu392Val substitution and a synonymous c1714t polymorphism in exon 12. SNP a1242t was not detected using TDI in 95 Caucasians. PCR-RFLP analysis for c1714t observed an allele frequency of 1% in 95 Caucasians, 0% in 71 Chinese, and 4.9% in 92 Ghanaians (p=0.002). This data highlights the importance of studying world populations when assessing DNA variations. CES2 SNP analysis is now being integrated into a polygenic pharmacogenetic approach for prediction of CPT-11 toxicity and efficacy in patients with cancer.

**Regulation of the tissue specific expression of the nephrin gene.** *O. Beltcheva*<sup>1</sup>, *S. Kontusaari*<sup>2</sup>, *H. Putaala*<sup>1</sup>, *K. Tryggvason*<sup>1</sup>. 1) Div. Matrix biology, MBB, Karolinska Inst., Stockholm, Sweden; 2) Biocenter and Dept. of Biochemistry, University of Oulu, Oulu, Finland.

The gene for nephrin, a key component of the kidney filter was recently cloned in our laboratory. Using *in situ* hybridization and performing detailed analyses of nephrin knockout mice Putaala *et al.* showed that the gene is expressed in kidney, brain and the pancreas. In kidney the nephrin expression is restricted to podocytes. While nephrin protein is proposed to have a major role for the urine filtration in kidney, its functions in brain and pancreas remain unknown. Studies of the regulatory mechanisms of the nephrin gene may help to clarify aspects of podocyte differentiation and biology and they may also allow us to better understand the extrarenal functions of nephrin.

In order to identify the nephrin promoter sequence and to distinguish between the transcription elements involved in its tissue specific expression we cloned 5 different segments of the 5' upstream region of nephrin in front of b-galactosidase as a reporter gene. The constructs were then used to generate transgenic mouse lines. Tissue expression of LacZ in the different mouse lines was studied by histochemistry. We also performed analyses of the nephrin mRNA in brain and kidney. The results from our experiments show that differential expression of nephrin is controlled by both alternative splicing in the 5' end of the gene and existence of tissue specific elements in the promoter.



**Characterization of a new SOX10 target gene in the glial lineage.** *M. Girard, N. Bondurand, V. Pingault, N. Lemort, M. Goossens.* INSERM U468, et Laboratoire de Biochimie et Genetique Moleculaire, AP-HP, Hopital Henri Mondor, Creteil, FRANCE.

The identification of the mammalian testis determination factor SRY led to the description of the SOX gene family, a new class of genes coding for transcription factors. The SOX genes are highly conserved through evolution and appear to govern cell fate during embryogenesis. Among them, SOX10 was identified as an essential factor in the enteric nervous system (ENS), melanocytes and glial cells development. Mutations in the SOX10 gene have been described in several cases of Shah-Waardenburg syndrome, a neurocristopathy characterized by the association of Hirschsprung disease (intestinal aganglionosis) and Waardenburg syndrome (pigmentation defects and sensorineural deafness). Some patients also present with myelination defects of the central and peripheral nervous system. In accordance, it has been shown that SOX10 controls expression of MPZ, MITF and RET, which play important roles during glial cells, melanocytes and ENS development, respectively. Nevertheless, it is likely that SOX10 regulates other genes, some of them involved in glial cells development. To test this hypothesis, we sought the possible involvement of SOX10 in the regulation of expression of several candidate genes, including MBP, PMP22 and GJB1 (Connexin32). Here we show that SOX10 strongly activates GJB1 expression in transfection assays by binding directly to the proximal region of the GJB1 promoter, while SOX10 mutant proteins fail to transactivate this promoter. Moreover, SOX10 is known to modulate the function of other transcription factors such as PAX3, EGR2 and POU3F1. We demonstrate in our in vitro system that these factors act in synergy with SOX10 on the GJB1 promoter. Overall, our results provide insights into the regulation of Connexin32 expression, and underline the potential importance of SOX10 during the myelination process. Our study may also improve our understanding of molecular mechanisms involved in the phenotypic features of some peripheral neuropathies.

***In vivo* DNase I footprint analysis of the kinin B1 receptor gene promoter shows footprints specific to cell types.**

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Nearly absent in healthy tissues under normal physiological conditions, the kinin B1 receptor (B1R) gene product is strongly and quickly upregulated following tissue injury and inflammation. Beside the participation of interleukin-1 beta (IL-1b), the molecular mechanisms implicated in this upregulation are not clearly defined. Gene promoters are best studied in their natural state in living cells and LMPCR (Ligation-Mediated Polymerase Chain Reaction) is the best approach to efficiently analyze DNA from living cells. *In vivo* DNA footprinting analysis of the B1R gene promoter region using dimethylsulfate and UVC was conducted on four human cell types: IMR-90, HEK-293, smooth muscle cells (SMC) and peripheral blood lymphocytes (PBL). The analysis revealed no difference between the footprinting patterns of the four cell types studied. Moreover, no additional change of the *in vivo* footprinting pattern was observed upon cell treatment with IL-1b. The footprinted sequences were analyzed using a transcription factor database and putative transcription factors were identified. In contrast to previous *in vitro* studies, no footprinted NF-kB site was found *in vivo*. To further characterize the promoter, *in vivo* DNase I footprinting analysis of the B1R gene promoter region was conducted on SMC and PCL. Although, no additional change of the *in vivo* footprinting patterns was found upon cell treatment with IL-1b, additional DNA sequences were footprinted upstream from the core promoter region. Interestingly, footprint differences were seen between PBL (cell expressing a non-functional B1R) and SMC (cell expressing a functional B1R). With over 1.4 kb of DNA analyzed in the promoter region, we can reasonably speculate that specific regulatory sequences are constitutively bound by transcription factors to ensure basal transcription and upregulation must be driven by protein cofactors, undetectable by *in vivo* DNA analysis.

**Characterisation of an erythroid-specific domain of histone acetylation across the  $\alpha$ -globin locus.** *C.A. Johnson<sup>1, 3</sup>, E. Anguita<sup>2</sup>, D.R. Higgs<sup>2</sup>, B.M. Turner<sup>1</sup>*. 1) Chromatin and Gene Expression Group, Anatomy Department, University of Birmingham Medical School, Birmingham, B15 2TT, UK; 2) Molecular Haematology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DS, UK; 3) Medical and Molecular Genetics, Birmingham Womens Hospital, Birmingham, B15 2TG, UK.

An important current issue in transcriptional regulation is the relationship between epigenetic phenomena, disease pathogenesis and the establishment, maintenance and inheritance of patterns of gene expression. Epigenetic control can be mediated by several types of chromatin modification and remodelling events that alter the structure of chromatin. Specifically, transcriptional activation and maintenance of an euchromatic state has a general correlation with post-translational acetylation of specific lysine residues in histone proteins.

To investigate the molecular events that link gene expression and the establishment of histone acetylation patterns, we have used the terminal 300kb of the short arm of human chromosome 16 (16p13.3) as a model system. This is a GC-rich telomeric region that contains a high density of unmethylated CpG-rich islands and widely-expressed genes, in addition to the  $\alpha$ -globin cluster. The embryonic and fetal/adult  $\alpha$ -like genes in this cluster are expressed in a defined tissue- and developmental stage-specific manner. To analyse patterns of histone acetylation at these genes and their regulatory elements, we have used the technique of chromatin immunoprecipitation (ChIP). In non-erythroid cells, there are moderate levels of all acetylated H4 histones across the  $\alpha$ -globin cluster, with low levels at heterochromatic and telomeric sequences. However, in erythroid cells there is a dramatic enrichment of the most highly acetylated histones (assayed with an antibody against histone H4 acetylated at lysine 5) in 100kb of chromatin that contains the  $\alpha$ -globin cluster and its regulatory element. This broad region of acetylation has sharp boundaries that may demarcate a complete chromosomal domain. Such domains are thought to be an important feature of genome organisation in eukaryotic chromosomes.

**Transcriptional regulation of murine matrix metalloproteinase 9 (MMP-9).** *E.E Hjörleifsdóttir*<sup>1</sup>, *S. Kontusaari*<sup>2</sup>, *K. Tryggvason*<sup>1</sup>. 1) MBB, Matrix biology, Karolinska Institute, Stockholm, Sweden; 2) Biocenter and Dept. of Biochemistry, University of Oulu, Oulu, Finland.

The aim of this project is to characterize the mechanisms that regulate tissue-specific expression of the MMP-9 gene. MMP-9 belongs to a large family of enzymes that are capable of degrading components of the extracellular matrix. These enzymes play a crucial role in many important biological processes, like embryonic development, bone remodeling, wound healing, and formation of blood vessels. Furthermore, MMPs are involved in tissue turnover in many pathological conditions; e.g. arthritis, tumor metastasis and tumor associated blood vessel growth. Prior to this study we have shown that a 5 kb long sequence upstream of the MMP-9 gene in mouse contains the regulatory elements necessary for tissue-specific expression in osteoclasts and migrating keratinocytes. In order to close in on the regulatory sequences, DNA-constructs containing different parts of the 5 kb sequence in front of a LacZ reporter gene, have been used to make transgenic mouse lines. So far four different mouse lines have been generated and the analysis of the reporter gene expression is ongoing. The whole region has been sequenced and the sequence analyzed both by searching databases for potential transcription factor binding sites and comparing the mouse sequence to the recently available human sequence. This computer analysis of the sequence gives further clues on where the regulatory elements may reside. The next step in the project will be to map the protein binding sites with direct methods, using methods like gel mobility shift assay, DNaseI hypersensitivity and Chromatin Immuno precipitation.

**Reduced expression of b<sub>2</sub>-glycoprotein I (apolipoprotein H) associated with a transcriptional initiation site mutation (-1C®A) that also disrupts the binding of hepatic nuclear factors.** *H. Mehdi, P. Desai, X. Wang, R. Zarnegar, S. Strom, M.I. Kamboh.* University of Pittsburgh, Pittsburgh, PA.

Human b<sub>2</sub>-glycoprotein I (b<sub>2</sub>GPI), also known as apolipoprotein H (apoH), is a subject of intense investigation, because it plays a central role in the production of antiphospholipid antibodies in the sera of patients with primary antiphospholipid syndrome and lupus. There is a wide range of interindividual variation in b<sub>2</sub>GPI plasma levels (<1 mg/dl to 35 mg/dl), and individuals with low b<sub>2</sub>GPI plasma levels are considered to be prone to thrombosis. The wide interindividual variation in b<sub>2</sub>GPI plasma levels is thought to be under genetic control, but its molecular basis is unknown. We have previously demonstrated that two polymorphisms in the coding region, Cys306Gly and Trp316Ser, are significant determinant of variation in b<sub>2</sub>GPI plasma levels. However, our *in vitro* mutagenesis and expression studies revealed that these mutations are not causative, but are in linkage disequilibrium with yet to be determined functional mutations. Therefore, we hypothesize that the 5' flanking region of b<sub>2</sub>GPI harbors functional mutations that determine interindividual variation in b<sub>2</sub>GPI plasma levels. To test our hypothesis, we screened 600 nucleotide region upstream of the transcriptional initiation site for mutation detection by D-HPLC. We identified a functional mutation at the transcriptional initiation site (-1C®A), which is associated with low b<sub>2</sub>GPI plasma and mRNA levels. We also found that the -1C®A mutation is associated with two-fold decrease in the luciferase reporter gene expression and it disrupts the binding of crude hepatic nuclear extracts as well as purified TFIID on electrophoretic gel mobility shift assay (EMSA). Screening of the b<sub>2</sub>GPI-deficient samples revealed that although the -1C®A mutation was associated with extremely low b<sub>2</sub>GPI plasma and mRNA levels, not all deficient samples had this mutation. Therefore, we conclude that the -1C®A mutation is one of the causative mutations and additional functional mutations may exist in the promoter region of b<sub>2</sub>GPI, which affect gene expression and ultimately plasma levels of b<sub>2</sub>GPI.

**Transcriptional regulation of the *GFRA1* gene.** *J.R. Hansford, S.L. Samson, D. Naumovski, J.B. Vanhorne, L.M. Mulligan.* Department of Pathology, Queen's University, Kingston, Ontario, Canada.

The glial cell line-derived neurotrophic factor receptor alpha-1 (GFRa-1) is an extracellular, non-signaling co-receptor for the RET receptor tyrosine kinase. GFRa-1 binds members of the glial cell line-derived neurotrophic factor (GDNF) family of ligands and presents them to RET. GFRa-1 has been shown to be expressed in cells of the central and peripheral nervous systems and in the kidney in a tissue-specific, developmentally regulated pattern. Mouse knock-out models have demonstrated that GFRa-1 is the RET co-receptor required for enteric neurogenesis and kidney development. In order to understand the mechanisms regulating the complex pattern of GFRa-1 expression observed in normal growth and development, we have studied the 5' upstream region of the gene, identified promoter(s) and transcriptional start sites and characterized tissue-specific transcriptional regulation of *GFRA1* expression. We have generated a sequence contig for the *GFRA1* 5' region and used this material to characterize the gene structure. Using a combination of 5' RACE and RT-PCR with total RNA from kidney and neuroendocrine cell types, we have identified three putative transcription start sites within our sequence contig. In order to confirm transcription from these sites, we generated a series of subclones from our contig, using the pGL3 luciferase based reporter system, and compared the relative luciferase expression associated with each construct in transient transfections. Our analyses suggest that *GFRA1* may have multiple distinct upstream regulatory sequences or promoters. In preliminary studies, we have identified the minimal promoter region required for transcription from each of our transcription start sites and have compared their activity in several cell types. Our data suggest that regulation of *GFRA1* transcription is complex and may require both common and cell-type specific regulatory elements. Continued investigation of the regulatory regions of *GFRA1* may help determine its role in development, growth and disease.

**Transcriptional regulation of the microsomal triglyceride transfer protein (MTP) gene by dietary cholesterol.** *J.*

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Microsomal triglyceride transfer protein (MTP) catalyses the transfer of lipids to apoprotein B in the assembly and secretion of triglyceride rich lipoproteins. The central role of MTP in lipoprotein assembly suggests that modulation of MTP activity may lead to changes in plasma levels of low-density lipoprotein (LDL), an important determinant for atherogenic risk. We have investigated the response of the human MTP gene promoter to dietary cholesterol. Truncated MTP promoter constructs linked to a luciferase reporter gene have been introduced by transient transfection into primary hepatocytes isolated from Syrian hamsters fed 0.24% cholesterol supplemented diets for 14 days prior to hepatocyte preparation. Expression from the MTP promoter was stimulated 3-4 fold in these cells compared with hepatocytes from control fed animals. It has been suggested that the response of MTP to cholesterol is mediated via two sterol response elements (SREs). Constructs deleting all previously identified transcription factor binding sites including both SREs show a 50% reduction in expression when compared to expression of the intact promoter in control cells. A 4-fold increase in expression, however, is maintained with cholesterol loading despite also deleting sites for hepatic nuclear factor (HNF)-1, activator protein-1 and HNF-4. We have identified two direct repeat elements with similarity to HNF-4 binding sites in the proximal MTP promoter. Co-transfection of an HNF-4 expression vector replicated the cholesterol response in both the intact MTP promoter and a construct containing only the two putative HNF-4 sites. Cholesterol loaded hepatocytes showed a greater increase in promoter activity with HNF-4 over-expression, implying that cholesterol facilitates the action of HNF-4. Expression of a dominant negative HNF-4 mutant reduced MTP expression in the presence and absence of over-expressed wild type HNF-4, suggesting that endogenous HNF-4 is normally required for MTP expression. Binding activities and the interaction of the HNF-4 sites in the MTP promoter are currently under study.

**Optimization of the cell membrane permeabilization for in vivo DNaseI footprint analysis.** *S. Ouellet, N. Dallaire, R. Drouin.* Med Biol, Faculty of Medicine, Hosp. St-Francois d'Assise, Quebec, Quebec, Canada.

Gene promoters are best studied in their natural state in the living cell and, thus, it is not surprising that in vivo DNA footprinting is one of the most accurate predictors of the state of transcriptional activity of genes. Ligation-Mediated Polymerase Chain Reaction (LMPCR) is the method of choice for in vivo footprinting and DNA structure studies because it can be used to investigate complex genomes, such as the human genome. In essence, LMPCR can be combined with a variety of modifying agents used for probing chromatin structure in vivo, including DNaseI. Compared to dimethylsulfate and UV, DNase I is less base selective, is more efficient at detecting minor groove DNA-protein contacts, provides more information on chromatin structure, displays larger and clearer footprints, and better delimits the boundaries of DNA-protein interactions. In vivo DNaseI footprint analysis is technically difficult to carry out because DNaseI cannot penetrate cells without previous cell membrane permeabilization. The membrane permeabilization presents problems of reproducibility. Furthermore, the permeabilization conditions may influence the ability of DNase I to detect DNA-protein interactions and special DNA structures. The purpose of our work was the optimization of the membrane permeabilization using either lysolecithin or Nonidet P40 to make it more reproducible without losing any DNA-protein interactions. We investigated the effect of the temperature (from 4C to 37C) and the duration (from 15 sec to 360 sec) of the treatment on permeabilization of human fibroblasts and lymphoblasts using either lysolecithin or Nonidet P40. We have found that the temperature had no significant effect on permeabilization of fibroblasts and lymphoblasts independently of the agent used. On the other hand, the permeabilization duration is very critical for fibroblasts whereas it does not affect the permeabilization efficiency of lymphoblasts. The permeabilization treatments with both agents did not produce any losses of DNA-protein interactions, independently of the temperature and duration of the treatments.



**Alterations in the SRY-box transcription factor *SOX1* in Cataract Patients.** *H.M. Prior*<sup>1</sup>, *R. Clarke*<sup>2</sup>, *E. Héon*<sup>3</sup>, *M.A. Walter*<sup>2</sup>. 1) Dept Biol, The King's University College, Edmonton, AB, Canada; 2) Depts. Ophthalmology and Medical Genetics, University of Alberta, Edmonton, AB, Canada; 3) Eye Research Institute of Canada, Department of Ophthalmology, University of Toronto, Toronto, ON, Canada.

Members of the SOX family of transcription factors contain a conserved DNA-binding domain, the SRY-box, which was originally identified in the product of the Sex-determining Region on the Y chromosome (SRY) gene. Human genetic disorders such as XY sex reversal, campomelic dysplasia, and Waardenburg-Hirschsprung disease are associated with mutations in various SOX genes. SOX1 function has been linked to proper expression of crystallin genes in the developing lens by studies done on chick and mouse eyes. In mouse, SOX1 deletion animals have small eyes and cataracts. We have therefore screened a panel of cataract patients for alterations in SOX1 coding sequence. Several polymorphisms were identified in patients and controls, including a six basepair duplication creating a glycine-valine duplication in the putative protein. A thymine to cytosine transition near the 3' end of the gene, generating a tyrosine to histidine substitution, was identified in affected members of a family with zonular cataracts that was not found in a panel of 120 control chromosomes. This substitution in the SOX1 coding sequence may be associated with cataract formation in this family. The functional consequences of this alteration are currently being investigated.

**Hes-1, a known transcriptional repressor, functions as a transcriptional activator for the human acid a-glucosidase gene in human fibroblast cells.** *B. Yan, N. Raben, P. Plotz.* Arthritis/Rheumat Br, Clin Ctr, NIAMS/NIH, Bethesda, MD.

Transcription factor Hes-1 is one of a family of basic helix-loop-helix proteins which play important role in myogenesis, neurogenesis, hematopoiesis, and sex determination. It has been demonstrated that Hes-1 is a transcriptional repressor for a number of known genes. We have previously shown that Hes-1 acts as a repressor that binds to a silencer element within intron 1 of the human acid a-glucosidase gene, which encodes a lysosomal enzyme for glycogen-breakdown. Deficiency of the enzyme results in Glycogen Storage Disease type II (Pompe syndrome), an inherited progressive muscle disease. In this study, we have determined its transcription activity in normal human fibroblast cells with same reporter gene system. Using constructs containing the DNA element that demonstrates repressor activity in Hep G2 cells, we found that this element functioned as an enhancer in human fibroblast cells. Electrophoretic mobility shift assay and super-shift assays with specific antibodies revealed that binding pattern of transcription factors Hes-1 and YY1 to the element in fibroblast cells is the same as in Hep G2 cells. Site-directed mutagenesis analysis showed that Hes-1 alone functioned as a transcriptional activator. Furthermore, co-transfection experiments with expression plasmid for Hes-1 significantly enhanced the activating effect of Hes-1. Thus, these data demonstrate for the first time that Hes-1, a known transcriptional repressor, can work as a transcriptional activator - on the acid a-glucosidase gene in human fibroblast cells. The finding expands the complexity of our understanding of Hes-1 and related basic helix-loop-helix proteins.

**Mutations of *QRX*, a Novel Retinal Homeobox Gene, Are Associated with Retinal Degeneration.** *Q. Wang*<sup>1</sup>, *S. Chen*<sup>2</sup>, *N. Esumi*<sup>1</sup>, *P. Swain*<sup>3</sup>, *H. Haines*<sup>4</sup>, *P. Mathers*<sup>5</sup>, *J. Heckenlively*<sup>6</sup>, *S. Jacobson*<sup>7</sup>, *E. Stone*<sup>4</sup>, *A. Swaroop*<sup>3</sup>, *D. Zack*<sup>1</sup>. 1) Johns Hopkins University, Baltimore, MD. 21287; 2) Washington University School of Medicine, St. Louis, MO. 63110; 3) University of Michigan, Ann Arbor, MI. 48105; 4) University of Iowa College of Medicine, Iowa City, IA. 52242; 5) West Virginia University School of Medicine, Morgantown, WV. 26506; 6) University of California-Los Angeles School of Medicine, Los Angeles, CA. 90095; 7) University of Pennsylvania, Philadelphia, PA. 19104.

**Purpose:** To identify transcription factors involved in regulating photoreceptor-specific gene expression. **Methods:** Yeast one-hybrid assays, Northern blot analysis, In situ hybridization, radiation hybrid mapping, EMSA, DNaseI footprinting, cell transfections, sequencing and sequence analysis. **Results:** Yeast one-hybrid screens of a bovine retina cDNA-Gal4 fusion library using elements (Ret-1 and BAT-1) from bovine rhodopsin promoter as baits yielded a number of putative positive clones. Among them was one encoding a novel paired-like homeobox gene, designated as *Qrx*. *Qrx* is highly and preferentially expressed in multiple retinal cell types. Bovine and human *QRX* share 91% identity at amino acid level. The homeodomain of human *QRX* is 93% identical to that of *RX/RAX*, a factor that has previously been shown to be essential for eye development. Although the in vitro target sites for *QRX* and *RX/RAX* are identical, namely the Ret-1 and BAT-1 sites, in transient transfection assays *QRX* and *Rx/Rax* preferentially transactivate Ret-1 and BAT-1 sites, respectively. *QRX* specifically increases the effect of *CRX* and *NRL* on Ret-1 by more than 10 fold. Human *QRX* consists of 3 exons and maps to chromosome 19. Three putative heterozygous mutations have been found in *QRX*, 2 in cone-rod dystrophy patients and 1 in an age-related macular degeneration patient, but not in normal controls. **Conclusion:** As a novel member of the transcription factor network operating in the retina, *QRX* appears to be important in maintaining normal retinal function.

**Tal1 binding to centromeric satellite DNA: A new role for Tal1 in transcription repression.** *J. wen*<sup>1</sup>, *T. Noguchi*<sup>1</sup>, *K. shigematsu*<sup>2</sup>. 1) LCB,NIDDK, NIH, BETHESDA , MD; 2) Department of cell and molecular biology, Lawrence Berkeley National laboratory, Cyclotron Road,Berkeley CA.

Tal1 is a bHLH transcription factor that plays a role in blood development, and is aberrantly expressed in T-ALL. Although Tal1 can function as an activator or repressor of transcription during hematopoiesis via its E-box binding motif and by interacting with transcription factor, GATA-1, and other coactivators or corepressors, few target genes for Tal1 have been identified. To elucidate target sites for Tal1 interaction, we use immunoprecipitation of genomic fragments bound to Tal1 in K562 erythroleukemia cell nuclear extracts. We found two Tal1 binding fragments consisting of multiple E-boxes overlapping a binding site for SATB1 (special AT-rich sequence-binding protein 1), that binds to the matrix attachment regions (MARs) of DNA and can act as a repressor. One of these fragments, EEGS, contained two E-boxes, CATTG and CATCTG, and a GATA binding site, specific for GATA-1 and GATA-2, within the SATB1 site. Chromatin immunoprecipitation confirmed that EEGS bound Tal1 in the chromatin of K562 cells. The EEGS core sequence with the two E-boxes, GATA motif, and SATB1 binding site, exhibited intrinsic Tal-1 dependent repression in reporter gene assays in K562 cells, but not in HeLa cells. Repression increased with increased expression of Tal1 and required the intact E-boxes and GATA motif for maximal affect. The EEGS sequence shares up to 98% homology with select satellite DNA (localizing particularly to 1q12 and 1q11), repetitive elements localizing to the centromere region of the chromosome. In addition to SATB1 binding to EEGS, we determined a direct interaction between Tal1 and SATB1, and overexpressing SATB1 in K562 cells can augment the transcriptional repression produced by the EEGS, both raising the possibility that Tal1 may recruit or stabilize SATB1 binding to selected satellite DNA to involve in the formation of heterochromatin in this region to maintain the repressed state of centromeric associated DNA. These data identify a subset of centromeric satellite DNA with Tal1 binding in erythroid cells, and suggests a new role for Tal1 in transcription repression.

**FMR1 expression in multiple tissues: post-mortem study of a male carrier of a premutation.** *F. Tassone<sup>1</sup>, R.J. Hagerman<sup>2</sup>, C. Greco<sup>3</sup>, E. Spector<sup>4</sup>, P.J. Hagerman<sup>1</sup>*. 1) Dept Biological Chemistry, University of California at Davis, Davis, CA; 2) M.I.N.D. Institute, University of California Davis Medical Center, Sacramento, California; 3) Department of Pathology, UC Davis Medical Center, Sacramento, California; 4) Department of Pediatrics, UCHSC, Denver, Colorado.

To understand the possible significance of inter-tissue heterogeneity to clinical involvement in fragile X syndrome, we have determined the CGG repeat size and FMR1 expression levels in multiple tissues from a 70 year old male carrier of a fragile X premutation. The proband, with a premutation of 135 CGG repeats in peripheral blood leukocytes, was debilitated by a neurodegenerative condition prior to his death. Our analysis of the CGG repeat in various tissues found no variation in repeat size. The lack of inter-tissue heterogeneity is supportive of somatic stability for premutation alleles. We have also analyzed the tissue-specific distribution of FMR1 mRNA in 13 different brain regions and in peripheral blood leukocytes. The range of FMR1 mRNA in various tissues was quite broad, with the highest expression levels found in superior temporal gyrus, hippocampus, frontal cortex and amygdala. FMR1 mRNA in peripheral blood leukocytes was 3.8 ( $\pm 0.13$ )-fold higher than normal, in agreement with previous observations for individuals with a premutation of  $>100$  CGG repeats. Immunocytochemistry revealed a deficit in FMRP expression, with 62 percent FMRP-positive lymphocytes, indicating post-transcriptional deficit. Neuropathologic examination showed the presence of discrete, eosinophilic intranuclear inclusions in neuronal and glial cells throughout the brain. The origin and composition of these inclusions are currently under investigation.>>.

**ID4 is both a transcriptional target of BRCA1 and a negative regulator of BRCA1 expression.** *P.L. Welch, R.M.G. Hernandez, M-C. King.* Departments of Genomic Sciences and Medicine, University of Washington, Seattle, WA.

In many sporadic breast and ovarian tumors, expression of BRCA1 protein is reduced. Identification of genes that regulate BRCA1 expression may elucidate the role of BRCA1 in sporadic tumorigenesis. ID4 (inhibitor of DNA binding 4) is a helix-loop-helix protein that functions as a dominant negative regulator of basic helix-loop-helix transcriptional regulators. ID4 is a negative regulator of BRCA1 expression. Modulation of ID4 expression resulted in inversely regulated BRCA1 expression; increased expression of ID4 was associated with anchorage-independent growth (PNAS 98:130, 2001). We analyzed genes regulated by BRCA1 in epithelial cell lines in which levels of BRCA1 were controlled by induction via ecdysone. We selected lines in which BRCA1 was modestly induced (~8-fold) over endogenous message, because gross over-expression of BRCA1 induces apoptosis. RNA from induced and uninduced cells was isolated and hybridized to Affymetrix expression arrays. In three independent experiments, expression of ID4 was consistently increased in response to BRCA1 induction ( $P=0.0025$ ). If in normal cells BRCA1 expression is regulated by ID4, which in turn regulates BRCA1, then loss of BRCA1 could result in loss of ID4. To determine if this regulatory loop is relevant to breast tumorigenesis, we stained primary breast tumors with antibodies to both ID4 and BRCA1 proteins. Normal breast epithelium stained positive for both BRCA1 and ID4. BRCA1 protein expression was reduced or absent in 46 of 79 primary tumors (58%). ID4 expression was dramatically reduced in 33 of these 46 BRCA1-deficient tumors, but in only 7 of 33 tumors with normal BRCA1 expression. Loss of BRCA1 and ID4 protein expression were highly correlated in the breast tumors ( $r^2=0.65$ ).

**DNAH3: characterisation of the full length gene and mutation search in patients with Primary Ciliary**

**Dyskinesia.** *J.-L.C. Blouin<sup>1</sup>, C. Gehrig<sup>1</sup>, D. Jeganathan<sup>2</sup>, L. Bartoloni<sup>1</sup>, C. Rossier<sup>1</sup>, G. Duriaux-Sail<sup>1</sup>, N. Scamuffa<sup>1</sup>, H.M. Mitchison<sup>2</sup>, C.D. DeLozier-Blanchet<sup>1</sup>, S.E. Antonarakis<sup>1</sup>.* 1) Division of Medical Genetics, University Cantonal Hospital and School of Medicine, Geneva, Switzerland; 2) Department of Paediatrics and Child Health, Royal Free and University College Medical School, University College London, United Kingdom.

Primary Ciliary Dyskinesia (PCD), or Immotile Cilia Syndrome, is an autosomal recessive disorder with an incidence of 1/20,000 characterized by dysmotility to complete immobility of cilia/flagella with ultrastructural defects in most patients. In addition to upper respiratory tract infections, bronchiectasis and male subfertility, 50% of cases show situs inversus (Kartagener syndrome). We have previously performed a genome-wide linkage analysis (Blouin et al. *Eur J Hum Genet* 2000 8:109-118) showing extensive genetic locus heterogeneity, as suggested by the variety of ciliary ultrastructural defects. However, we proposed several potential loci on chromosomes 3p, 5p, 8q, 11p, 15q, 16p, 17q and 19q, most of them colocalizing with genes coding for dyneins, the major structural proteins of dynein arms defective in 50% of PCD families and therefore strong candidates for the disease. Since then, mutations in dynein genes DNAI1 and DNAH11 have been detected in patients. Our most suggestive/almost significant linkage interval on chromosome 16p near marker D16S748 (NPL score =2.96 on families with dynein arm deficiency) contains the gene for axonemal dynein heavy chain DNAH3. We report here full length sequence characterisation of DNAH3 gene and mutation search in patients with PCD. Genomic and transcript organisation of the gene were determined using cDNA isolated by RT-PCR and searches of genomic sequences and ESTs. The DNAH3 gene spans 200 Kb of genomic DNA and is composed of approximately 60 exons coding for a protein of 4400 amino acids, highly homologous to known dynein heavy chain paralogues and orthologues. Mutation search, currently under progress, is performed in selected patients, with the double occurrence of allele sharing among affected sibs using a polymorphic marker close to the gene and ultrastructure defects of dynein arms.

Program Nr: 1082 from the 2001 ASHG Annual Meeting

**The alternative splicing of DMPK is affected *in cis* by the CTG expansion of Myotonic Dystrophy Type-1..** A.J. Gibb, M.G. Hamshere. School of Life & Env. Sciences, University of Nottingham, Nottingham, Nottinghamshire, UK.

Myotonic Dystrophy Type-1 (DM1) is the most common muscular dystrophy affecting 1/8000 live births in Western European and North American populations. The condition is characteristically multisystemic with symptoms including myotonia, progressive muscle weakness, cardiac conduction defects, cataracts, frontal balding in males, testicular atrophy, diabetes, hypersomnia and mental retardation. The condition segregates in an autosomal dominant manner and is caused by a CTG triple-repeat expansion found in the 3'UTR of the gene for a serine-threonine protein kinase, DMPK.

*DMPK* has several alternative splice forms. The frequency of their use and the nature of any effect mediated by the repeat, either *in cis* or *in trans*, has not yet been fully investigated. Using affected and control fibroblasts heterozygous for a SNP in Exon 10 of *DMPK*, we have been able to develop a fluorescence based quantitative RT-PCR method in order to discriminate between transcripts derived from the affected and unaffected chromosomes. By a combination of fluorescence and size discrimination, we have established that the profile of alternative splicing is affected *in cis* by the myotonic dystrophy triplet repeat expansion.



**Identification of alternatively spliced messengers for IL-12p35 and IL-12Rb2 genes.** *H. Elloumi Zghal, S. Abdelhak, M.R. Barbouche, K. Dellagi.* Immunology Dept, Institut Pasteur, Tunis, Tunisia.

Interleukin 12 is a disulfide heterodimeric glycoprotein comprised of two subunits designated p35 and p40. This pleiotropic cytokine is produced primarily by antigen-presenting cells. IL-12 plays a pivotal role in promoting cell mediated immunity against intracellular pathogens as it is a dominant factor in directing the development of Th1 cells producing high levels of IFN-g. These immune responses are mediated through its interaction with a high affinity cell surface receptor (IL-12R) on activated T and NK cells composed of at least two subunits termed b1 and b2. In the present study, we have identified alternatively spliced mRNA variants for IL-12p35 and IL-12Rb2 genes. For IL-12p35 gene, three transcripts including the normal one have been identified. One alternative transcript corresponds to exon 2 skipping generating a premature stop codon at position 300-302. The other messenger shows an in-frame deletion of exon 3 resulting in an isoform deleted for 38 amino acids. For IL-12Rb2 gene, subcloning of RT-PCR products allowed the identification of a normal as well as an alternatively spliced transcript missing exons 4 to 7. Furthermore, the latter is sometimes associated to an insertion of 102bp between exons 8 and 9. This inserted sequence corresponds to the use of cryptic splice sites within intron 8 and shows a homology of 91% with Alu Sx subfamily. These isoforms deleted for the cytokine receptor-homologous region (CHR) may correspond to a diminished ligand binding capacity. Our findings may suggest that the identified alternatively spliced transcripts of IL-12p35 and IL-12Rb2 genes correspond to regulatory forms that diminish the ligand-receptor interaction. Such post transcriptional mechanism may contribute to the host protection against the deleterious effects of IL-12 over-production.

**A 16-bp duplication of splice-acceptor site results in silencing of the downstream copy of the splice-acceptor site.**

**How are duplicated splice-acceptor sites selected in pre-mRNA splicing?** *K. Ohno<sup>1</sup>, A. Tsujino<sup>1</sup>, B. Anlar<sup>2</sup>, A.G. Engel<sup>1</sup>*. 1) Dept Neurology, Mayo Clinic, Rochester, MN; 2) Hacettepe University, Ankara, Turkey.

Congenital myasthenic syndromes (CMS) are caused by genetic defects in presynaptic, synaptic, or postsynaptic molecules. In a CMS patient, we identified a homozygous 16-bp duplication of the splice-acceptor site at the intron 10/exon 11 boundary of the acetylcholine receptor  $\epsilon$  subunit gene (eIVS10-9ins16). The duplication consists of 8 nucleotides at 3' end of intron 10 ('cccgccag', introns are shown in lower case letters) and 8 nucleotides at 5' end of exon 11 ('CTGCCTTC', exons in capital letters).

Wild type: ctgccaggggggaggctcctaaggcccaccccggtgtttccccgccagCTGCCTTC

eIVS10-9ins16: tcctaaggcccaccccggtgtttcccgcagCTGCCTTCcccgccagCTGCCTTC

To test which of the duplicated splice-acceptor sites is activated in pre-mRNA splicing, we constructed a minigene that spans exons 9 to 12 of the acetylcholine receptor  $\epsilon$  subunit gene and introduced it in COS cells. RT-PCR analysis of cytoplasmic RNA of transfected COS cells revealed that only the upstream copy of the splice-acceptor site is active. Although the duplication disrupts the polypyrimidine tract of the downstream copy of splice-acceptor site, the pyrimidines-to-purine ratio remains unchanged in the polypyrimidine tract. However, a 'tttt' stretch at positions -13 to -10 of the upstream copy is changed to 'cctt' for the downstream copy. As a stretch of t's is more efficient than c's in the polypyrimidine tract, we mutated 'cctt' to 'ttt', but the downstream copy still remained inactive. To test a hypothesis that a distance between the branch point sequence and the 'ag' dinucleotide is important, we moved the invariant 'a' in the 'tcctaag' branch point sequence 15 bp downstream to make a new branch point sequence of 'cggtgat' in both wild-type and mutant minigenes. These mutations, however, had no effect on splicing. Further mutagenesis studies are in progress to identify essential *cis*-acting elements that govern selection of the duplicated splice-acceptor sites.

**Human L1 ORF2 is translated in an unconventional manner.** *R.S. Alisch, J.V. Moran.* Human Genetics & Internal Med., Univ. of Michigan, Ann Arbor, MI.

LINES (L1s) are the most abundant retrotransposons in the human genome. Retrotransposition-competent L1s are 6.0 kb and contain a 5' untranslated region (UTR), two non-overlapping open reading frames (ORF1 and ORF2), and a 3' UTR that ends in a polyadenylic acid tail. It previously was demonstrated that certain L1s could retrotranspose in cultured human cells and that mutations in conserved domains of the ORF1- and ORF2-encoded proteins (ORF1p and ORF2p) block retrotransposition. To identify cis-acting sequences in L1 RNA important for ORF2 translation, we performed site-directed mutagenesis to alter conserved bases in the vicinity of the putative ORF2 initiation codon. Frameshift mutations positioned upstream of the initiation codon had virtually no effect on retrotransposition, whereas the introduction of a stop codon (L11X) near the presumptive amino terminus of ORF2p completely abolished retrotransposition. Remarkably, missense mutations in the putative initiation codon (AUG to AUA; AUG to CCC) only slightly reduced retrotransposition (to ~30% of wild-type levels). To prove that endogenous sources of reverse transcriptase (RT) were not promoting the retrotransposition of these constructs, we created a double mutant that contains the AUG to CCC mutation as well as a point mutation in the RT domain of L1 ORF2. This double mutant cannot retrotranspose, indicating that the ORF2p is synthesized in the original mutants. To further demonstrate that ORF2p was synthesized in our full-length L1 constructs, we fused a luciferase reporter gene to ORF2. Luciferase activity was detected in HeLa cells transfected with retrotransposition-competent constructs (~10-fold above background levels). By contrast, cells transfected with retrotransposition-defective mutants (e.g., L11X) exhibited only background levels of activity. Because there is no evidence for splicing of L1 mRNA, and there are no other candidate initiation codons (AUG, GUG, CUG) near the presumptive amino terminus of L1 ORF2, our data suggest that L1 ORF2 translation may be initiated from an unconventional (non-AUG) codon.

**Human KPNA2 regulation of cell cycle and tumorigenesis pathways.** *B.A. Fullmer, K. Lieuallen.* Research and Development, Gene Logic Inc., Gaithersburg, MD.

Karyopherin alpha 2 (KPNA2) belongs to a family of importins that are receptors for nuclear localization signals implicated in nuclear transport systems. Importins are responsible for the initial docking at the nuclear pore complex and are also associated with the interactions that occur within the pore complex during translocation. KPNA2 has been associated with cellular functions ranging from proliferation to viral susceptibility. To determine the specific pathways associated with KPNA2, transfection studies were performed with KPNA2-specific antisense oligonucleotides (ASOs) on human umbilical vein endothelial cells (HUVEC). Cells were treated with either KPNA2-specific ASO, a control missense ASO, or the transfection reagent alone (vehicle control). Total RNA from each cell line was hybridized over the Affymetrix known gene array (12,000 known genes, HU 60K sub A), to assess global transcriptional changes as a result of loss of KPNA2 mRNA expression. Genes that were regulated in both sets of transfection assays were examined and assigned to biochemical/physiological pathways. A total of 268 genes out of 12626 genes showed significant differences in expression levels between cells treated with the ASO and vehicle control. Of these genes, 39 showed a significant difference with a p value of less than or equal to 0.01 in the antisense oligo versus the missense and vehicle control. Seven of these genes are known to participate in the cell cycle while four are known to be implicated in tumorigenesis.

**Isolation, expression, and subcellular localization of the mouse *Tmprss3*.** *M. Guipponi*<sup>1</sup>, *K. Shibuya*<sup>2</sup>, *M. Vasquez*<sup>3</sup>, *L. Dougherty*<sup>4</sup>, *N. Scamuffa*<sup>1</sup>, *E. Guida*<sup>5</sup>, *M. Okui*<sup>2</sup>, *C. Rossier*<sup>1</sup>, *P.L. Marzella*<sup>5</sup>, *N. Shimizu*<sup>2</sup>, *H.S. Scott*<sup>4</sup>, *S.E. Antonarakis*<sup>1</sup>. 1) Div Medical Genetics, University of Geneva Medical School, Switzerland; 2) Dpt of Molecular Biology, Keio University School of Medicine, Tokyo, Japan; 3) Dpt of Morphology, University of Geneva Medical School, Switzerland; 4) Genetics and Bioinformatics group, The WEHI, Royal Melbourne Hospital, Australia; 5) Department of Otolaryngology, The University of Melbourne, Australia.

Recently, we have found that the human *TMPRSS3* gene is responsible for two previously defined non-syndromic autosomal recessive deafness located on human chromosome 21q22.3, DFNB8 and DFNB10 (Scott et al Nat Genet 27:59, 2001). *TMPRSS3* encodes a putative protein that contains 4 recognizable domains: transmembrane-LDLRA-SRCR-Serine protease. Several loss-of-function mutations have been identified in families with these forms of deafness. In order to investigate the expression profile and the function of the *TMPRSS3* protein, we isolated the mouse homologue (*Tmprss3*), and determined its expression profile and subcellular localization. The mouse *Tmprss3* gene is split into 13 exons over 20 kb of genomic sequence on chromosome 17 and encodes a predicted protein that shows 95% identity with the human protein. *Tmprss3* expression was detected by RT-PCR in rat modiolus, stria vascularis, organ of Corti and cultured spiral ganglion cells. RNA in situ hybridization on mouse embryos ranging from 8.5 to 10.5 dpc, and on inner ear sections derived from 5-day postnatal mice showed *Tmprss3* expression in the supporting cells the organ of Corti and in the spiral ganglion. The subcellular localization of EGFP-*TMPRSS3* fusion protein in transfected HeLa cells was found diffuse in the cytoplasm; colocalization experiments suggested that *TMPRSS3* is an endoplasmic reticulum protein. These data provide first insights toward the definition of the precise role of *TMPRSS3* in the function of the inner ear. The generation of a mouse with targeted disruption of the *Tmprss3* gene is in progress; this will provide an animal model for the elucidation of the molecular pathophysiology of DFNB8/10.

**Analysis of mutations in Survival of Motor Neuron (*SMN1*) gene through model systems.** *N. Owen*<sup>1</sup>, *S.E. Squire*<sup>1</sup>, *U.R. Monani*<sup>2</sup>, *M. Sendtner*<sup>3</sup>, *A.H.M. Burghes*<sup>2,4</sup>, *K.E. Davies*<sup>1</sup>. 1) Dept of Human Anatomy & Genetics, University of Oxford, Oxford, England, UK; 2) Dept of Neurology, College of Biological Sciences, Ohio State University, Columbus, OH; 3) Dept of Neurology, University of Würzburg, Josef-Schneider Strasse 11, Würzburg, Germany; 4) Dept of Molecular Genetics, College of Biological Sciences, Ohio State University, Columbus, OH.

Childhood onset Spinal Muscular Atrophy (SMA) is a common autosomal recessive disorder characterised by the loss of a motor neurons. The Survival Motor Neuron (*SMN1*) gene, present in two almost identical copies on chromosome 5, is mutated in the majority of patients. Both *SMN* genes undergo varying degrees of alternative splicing, with *SMN1* producing mainly full length transcript, as compared to *SMN2* whose expression results in predominately alternatively spliced transcripts. Therefore, in patients the amount of full length SMN protein is markedly reduced. *SMN2* copy number correlates strongly with disease severity; more copies resulting in increased SMN protein and a milder phenotype. To further understand the effect of patient *SMN1* mutations and alternative splicing of *SMN2* we have characterised mutant SMN proteins. To specifically investigate loss of exon 5 we have generated transgenic mice expressing *SMN2D5*. A second transgene utilises the E134K patient mutation known to alter interaction of SMN with Sm proteins. The ability of each transgene to rescue the lethality of the null mouse has been investigated. Up-regulation of *SMN2* may act as a potential therapeutic route, therefore the effects of increasing spliced *SMN* isoforms needs to be investigated.

Previously, analysis of our model system in *S.pombe* has lead to the identification of a lethal missense mutation in a conserved region of the SMN protein. This conserved domain is in human exon 5. Proteins known to interact with this region of SMN are profilins. *In vitro* assays have been carried out to investigate the effect of mutation this domain on binding to PFNs. The generation of transgenic models utilising *SMN* mutations and binding assays will provide further insight into the pathogenesis of SMA.

**Characterization, tissue expression, and chromosomal assignment of the human RAB22A gene belonging to Rab small GTPases.** *S. Kussmann, S. Worch, A. Hehr, I. Hansmann, D. Schlote.* Institut fuer Humangenetik und Medizinische Biologie; Halle, Germany.

The mouse chromosome 2 segment (MMU2) corresponding to human chromosome 20 (HSA20) is known to be involved in both, maternal as well as paternal noncomplementation (genomic imprinting). Uniparental disomies for distinct regions of MMU2 result in different neonatal lethalties with opposite anomalous phenotypes, strongly suggesting the presence of imprinted genes in this region. These chromosomal regions show a conserved synteny of gene loci to human 20q13 segment, predicting the presence of imprinted genes in this syntenic human chromosomal region. We have identified a new gene in this region of interest which is located on a BAC RPCIB753L051096 proximal to GNAS1 on HSA 20q13. Cloning and sequencing the full-length cDNA revealed a novel isoform of the human RAB22 subfamily of small GTP-binding proteins located in distinct intracellular compartments and playing an important role in the regulation of vesicular trafficking. Based on the EST WI-12997 this new isoform was isolated containing 2242 nucleotides and is designated RAB22A. Structurally, the RAB22A encodes a polypeptide of 194 amino acids which has 97% identity to the canine rab22. Northern blot analysis revealed ubiquitous expression slightly increased in heart. The genomic structure was completed by database analysis and sequencing of the isolated BAC clone RPCIB753L051096. The gene consists of 7 exons spanning about 50 kb of genomic sequence. Physical and FISH mapping revealed that RAB22A is located on a BAC RPCIB753L051096 proximal to GNAS1 but downstream of PCK1 on human chromosome 20q13. Supported by a grant of the DHGP.

**PITX2 homeodomain protein forms a dimer: Insights from natural and synthetic mutations.** A. Kuburas<sup>1</sup>, I. Saadi<sup>2</sup>, A.F. Russo<sup>1,2</sup>. 1) Department of Physiology and Biophysics; 2) Genetics Program, University of Iowa, Iowa City, IA.

Rieger syndrome is an autosomal-dominant human disorder characterized by glaucoma, mild craniofacial dysmorphism, umbilical stump abnormalities, and occasional growth abnormalities. Knockout studies have shown that mouse *Pitx2* is required for development of multiple organs, including the pituitary. We have previously described a mutation in a Rieger syndrome patient that changes a key lysine at position 50 of the homeodomain to a glutamic acid (K88E). This mutation yields a dominant negative protein and this phenotype is most prominent in the presence of the pituitary transcription factor Pit-1. We now provide evidence that this dominant negative effect is mediated by dimerization of K88E with wildtype PITX2 in the yeast two-hybrid assay. The significance of this dimerization is supported by the finding that a different Rieger homeodomain mutant (T68P) that is not dominant negative showed much weaker dimerization activity. The K88E mutant may possibly interact with portions of PITX2 to a greater extent than the wildtype interaction. These results suggest that the position 50 residue in the PITX2 homeodomain may play a role in facilitating PITX2 dimerization and the subsequent dominant negative phenotype of the K88E Rieger mutation.



**Genomic characterisation of the human PRNP/PRND locus.** *E. Makrinou*<sup>1</sup>, *J. Collinge*<sup>1</sup>, *M. Antoniou*<sup>2</sup>. 1) MRC Prion/Neurogenetics Unit, Imperial College School of Medicine, London, England; 2) Division of Medical and Molecular Genetics, GKT Medical and Dental School, Kings College, London, England.

Chromatin domains can consist of a mixture of genes, with some been ubiquitously expressed and others been tissue specific. Although the PRNP gene is found to be ubiquitously expressed, the levels of expression in a given tissue can vary markedly, with highest amounts observed in neurons and lymphocytes. In addition, Northern blot analysis of two genes laying within close distance, PRND and a second novel gene downstream of PRND, showed that they are both tissue specific. Here we report the genomic characterisation and expression profile of these two genes. Regulatory elements within the same domain may be shared with genes of similar or divergent characteristics, like PRNP and PRND. These findings may provide a new insight, regarding the presence and further study of any regulatory elements and/or a Locus Control Region (LCR), within the domain.

**Mutations Generating a Premature Stop Codon in the Protein S Gene (*PROSI*) Lead to Nonsense-Mediated mRNA Decay and Protein S Deficiency *PROSI*.** X. Munyoz, Y. Espinosa-Parrilla, N. Sala. Medical.Molecular Genetic Center, IRO, L'Hospitalet de Llobregat, Barcelona, Spain.

The purpose of this study was to analyse the effect at the mRNA level of different nonsense and frameshift mutations in the protein S gene (*PROSI*) that segregate with quantitative protein S (PS) deficiency in Spanish families with thrombophilia. **Methods:** Platelet *PROSI* mRNA was obtained from healthy controls and from 9 PS deficient patients who were heterozygotes for a missense mutation (M599T) and for 8 mutations postulated to generate a premature stop codon. Four of these are nonsense (Q238X, S293X, R410X, W465X), two are short frameshift deletions (333,334delTG and 1877delT), one alters the splice site (1302+5G->A) and one is a missense and a splice site mutation (404,405AG->GT). *PROSI* cDNA fragments covering *PROSI* exons 1 to 15 were obtained by RT-PCR and directly sequenced. The results obtained were confirmed by analysis of transcribed polymorphisms for which the patients were heterozygotes. **Results:** Electrophoretic analysis of the amplified cDNA fragments revealed the presence of normal-sized fragments in all cases, thus excluding splicing alterations. cDNA corresponding to both the normal and the mutated alleles was only observed from the M599T missense mutation in exon 15 and from the 333,334delTG mutation in exon 2. In the other 7 mutations analysed, the amplified cDNA sequence corresponded to that of the normal allele, indicating the absence (or presence in inappreciable quantities) of transcripts from the mutated allele. **Conclusion:** From these results we concluded that nonsense-mediated mRNA decay, rather than the synthesis of a truncated protein, is the main cause of quantitative PS deficiency associated to mutations which generate a premature stop codon.

We thank DGICYT (PB94/1233) and FIS (98/0645) for grants.

**A candidate gene for FSHD within each of the 3.3 kb repeated elements of the 4q35 locus.** C. Mattéotti<sup>1</sup>, J. Gabriëls<sup>2</sup>, J.M. Saint-Remy<sup>2</sup>, R.R. Frants<sup>3</sup>, G. Padberg<sup>4</sup>, D. Collen<sup>2</sup>, F. Coppée<sup>1</sup>, A. Belayew<sup>1,2</sup>. 1) Lab. Molecular Biology, University of Mons-Hainaut, Mons, Belgium; 2) CMVB, University of Leuven, Leuven, Belgium; 3) MGC Dept Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 4) Dept Neurology, University of Nijmegen, Nijmegen, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is linked to partial deletions of a tandem 3.3 kb repeat array in the heterochromatic D4Z4 locus: the repeat copy number decreases from 10-100 in non-affected individuals down to 1-8 in patients. We have identified a putative double homeobox gene (*DUX4*) in each of the two 3.3 kb repeats left in the rearranged D4Z4 locus of a patient (Gabriëls et al, 1999, Gene 236: 25-32). Functionality of this gene was questioned because the TATAA box is mutated to TACAA, and it lacks intron and a polyadenylation signal. We tested *DUX4* activity *in vivo* by injecting a plasmid with the 13.5 kb *EcoRI* DNA fragment of the patient locus into a mouse leg muscle. An active gene would express a human protein, triggering synthesis of antibodies in the mouse. Indeed, a strong immunoreactivity was detected in the sera of the 6 injected mice by ELISA using plates coated with part of *DUX4* expressed in *E. coli* as an MBP fusion. A much weaker response was found against MBP alone or in the preimmune sera.

A northern blot identified a 2.5- and a 1.3-kb *DUX4* RNA in total RNA extracted from mouse C2C12 cells transfected with this plasmid. The protein intracellular location was studied in cells transfected with a CMV-*DUX4* expression vector: a rabbit serum directed against the homeodomains detected the *DUX4* protein at the nuclear envelope, partially overlapping with lamin B and emerin.

In conclusion, we think *DUX4* is a new candidate gene for FSHD. We hypothesize that in non-affected individuals, the *DUX4* genes are in a repressive heterochromatin. The deletion linked to FSHD would open this chromatin and allow expression of the *DUX4* genes present in the few repeats left, producing a nuclear envelope protein toxic to muscles. (Supported by the AFM-France, the FSH Society, the MDA, and the G. Shaw family).

**Aggregation of misfolded proteins is a selective and sequence-specific process that determines the composition and cellular distribution of aggregates.** *M.I. Milewski<sup>1</sup>, J.E. Mickle<sup>1</sup>, J.K. Forrest<sup>1</sup>, B.A. Stanton<sup>2</sup>, G.R. Cutting<sup>1</sup>.* 1) Inst Genetic Medicine, JHU Sch Med, Baltimore, MD; 2) Dept Physiol, Dartmouth Med Sch, Hanover, NH.

Abnormal protein aggregation is associated with growing number of inherited and sporadic genetic diseases. However, little is known about relationship between the sequence of aggregating peptides and the process of intracellular accumulation. To test the specificity of protein aggregation, we examined the cellular localization and composition of aggregates formed by different aggregation-prone proteins. We show that CFTR- and huntingtin-derived constructs accumulate in separate aggregates when co-expressed in the same cell, which indicates that aggregation of misfolded proteins is a selective process. We also demonstrate that fusion to a reporter protein considerably alters the distribution of aggregating peptide. When fused to green fluorescent protein (GFP), the peptide containing amino acids 1370-1480 of CFTR accumulates in large perinuclear or nuclear aggregates. The same CFTR fragment devoid of GFP localizes predominantly to discrete accumulations associated with mitochondria. Importantly, both types of accumulation are dependent on the presence of the same two amino acids within the CFTR sequence. The inability of different misfolded proteins to co-aggregate or form a specific type of aggregates is accompanied by differences in association with molecular chaperones, which suggests a possible role for chaperones in regulating the pattern of protein accumulation. Together, our results contradict the common view that protein aggregates are non-specific associations of misfolded molecules. Instead, we propose that sequence- and structure-specific interactions are critical for protein aggregation, thus suggesting potential avenues for preventing aggregate formation in disease states.

Program Nr: 1095 from the 2001 ASHG Annual Meeting

**Targeted Disruption of the *Pcyt1b* Gene by Homologous Recombination.** *M.A. Karim, J. Wang, S. Jackowski.*  
Protein Science Division, Department of Infectious Diseases, St. Jude Children's Res Hosp, Memphis, TN.

The mouse *Pcyt1b* gene encodes two isoforms, CCTb1 and CCTb2, of the second CTP:phosphocholine cytidylyltransferase, a key regulator of phosphatidylcholine biosynthesis in mammals. We cloned a 9-kb genomic *Pcyt1b* gene fragment by screening a mouse 129/SvE library in IEMBL3 with a homologous human 5' *PCYT1B* cDNA probe. The isolated genomic clone contained the entire exon 1 plus flanking regions. The *Pcyt1b* gene is located on the X-chromosome and we disrupted the gene in male (XY) embryonic stem cells W9.5, resulting in a (-/0) genotype. Four individual stem cell clones with bona fide karyotypes were injected into blastocysts and clone #25 gave rise to chimeric males with germline expression of the disrupted allele. Agouti male offspring from the clone #25 chimeras did not carry the disrupted allele, as expected, whereas female agouti mice were heterozygous. CCTb-deficiency apparently did not alter male reproductive function, despite high-level expression in the testes of the wild-type allele. Heterozygous females will be mated with wild-type C57Bl6 males to obtain male mice hemizygous for the disrupted allele, which will be analyzed for the CCTb-deficient phenotype and also will be mated with heterozygous females to obtain (-/-) female mice. The availability of the *Pcyt1b* (-/0) and (-/-) mice will be useful for the investigation of the role of this gene in phospholipid metabolism, particularly X-linked disorders. (Supported by NIH GM45737, ALSAC and Cancer Center CORE grant to St. Jude Children's Research Hospital.).

**Two apparently unrelated proteins encoded by three related mRNAs having monocistronic and dicistronic structures.** *M. Takagi*<sup>1,2</sup>, *R. Sakaguchi*<sup>1</sup>, *Y. Tohyama*<sup>2</sup>, *J. Yamada*<sup>1</sup>, *T. Watanabe*<sup>1</sup>, *T. Kurihara*<sup>2</sup>, *T. Suga*<sup>1</sup>. 1) Dept. of Clinical Biochemistry, Tokyo University of Pharmacy and Life Science, Tokyo, Japan; 2) Institute of Life Science, Soka University, Tokyo, Japan.

We isolated three related cDNAs, C184L (2.5 kb), C184M (1.2 kb), and C184S (0.7 kb) from a developing mouse brain cDNA library. C184S is the 5'-end portion and C184M is the 3'-end portion, respectively, of C184L. C184S and C184M are monocistronic structures having open reading frames of 199 amino acids (ORF1) and 189 amino acids (ORF2), respectively; C184L has both ORF1 and ORF2 (dicistronic structure). Although the corresponding mRNAs were expressed ubiquitously in the tissues examined, C184L and C184M mRNA expressions were relatively high in the embryonic brain. Southern blot analysis suggests that all of the three related mRNAs are transcribed from the same single gene. The intervening region of C184L cDNA between ORF1 and ORF2 contained a promoter sequence for C184M mRNA, which is transcribed from the corresponding genomic sequence. Our results indicate that the mRNAs encoding these apparently unrelated proteins are transcribed within an adjacent or overlapping area on the genome, suggesting the same origin of the two transcription units. A cDNA (Sjögren's syndrome/scleroderma autoantigen 1, human autoantigen p27) encoding human homologue of ORF1 was isolated by immunoscreening using autoimmune antiserum from a patient with anti-centromere antibodies; a cDNA encoding a different mouse isoform of ORF2 was cloned as a novel mammary tumor virus receptor. The normal functions of both ORF proteins remain unclear. Cloning, genomic structure, and expression analysis of the human counterparts are ongoing, and could be an important step to understand their physiological functions and potential association with Sjögren's syndrome and scleroderma.

**Characterization of brain acyl-CoA hydrolase (BACH) gene.** *J. Yamada, Y. Kuramochi, R. Sakaguchi, T. Watanabe, M. Takagi, T. Suga.* Dept. of Clinical Biochemistry, Tokyo University of Pharmacy and Life Science, Tokyo, Japan.

Acyl-CoA hydrolases cleave acyl-CoA thioesters to free fatty acids and coenzyme A. The potency of this enzyme may be a mechanism that modulates cellular concentrations of acyl-CoAs to regulate various cellular events, including fatty acid metabolism and gene expression, for which only limited evidence is available. To better understand the physiological function of this enzyme, we characterized the brain acyl-CoA hydrolase (BACH) gene. Human BACH gene (1p36.31-p36.11) spanned about 130 kb and comprised 13 exons, generating several isoforms through a mechanism of alternative use of exons. Four first exons (1a-1d) can alternatively be used and three patterns of splicing occur at an exon X located between exons 7 and 8 that contains an internal 3'-splice acceptor site. There were motives for mitochondrial targeting sequence encoded in exons 1b and 1c, and a nuclear localization signal in exon 9. Real-time quantitative RT-PCR revealed that among the isoforms, BACHa with a sequence corresponding to exon 1a is exclusively expressed in the human brain, with or without 73- or 165-bp inserts derived from exon X that create premature stop codons. Similar results were obtained for mouse homolog of BACH gene, with respect to the genomic organization and the isoform expressed in the brain. Mouse BACHa cDNA encoded a 338-amino acid polypeptide with 96% identity to human BACHa, indicating that the BACH gene is highly conserved beyond the species. While palmitoyl-CoA hydrolase activity was widely distributed in mouse tissues, it was marked in the brain, consistent with BACH almost exclusively found there, where >80% of the enzyme activity was explained by BACH present in the cytosol. Immunohistochemistry demonstrated neuronal localization of BACH both in the central and peripheral nervous systems. A 50-kDa polypeptide along with the major 43-kDa polypeptide seemed to be translated from the BACHa mRNA using differential in-frame ATG triplets as the initiation codon. These findings will make a large contribution to the functional analysis of BACH gene including genetic studies.

**Identification and characterization of a novel Strongly Expected Brain Specific Transcript (SEBS1) as candidate for imprinting.** *S. Worch, S. Kussmann, A. Hehr, I. Hansmann, D. Schlote.* Institut fuer Humangenetik und Medizinische Biologie; Halle, Germany.

Uniparental disomies for distinct regions of mouse chromosome 2 result in opposite anomalous phenotypes, strongly suggesting the presence of imprinted genes in this region. These chromosomal regions show a conserved synteny of gene loci to human chromosome 20q13 segment, predicting the presence of imprinted genes in this syntenic human chromosomal region. Starting out from the human EST marker A005Z17 we have identified a novel transcript within the candidate region which is strongly expected to be brain specific as Northern hybridisation revealed expression of a 3,2 kb transcript in brain only. The corresponding cDNA contains a 1680 bp ORF encoding 559 amino acids. The genomic structure was determined by database analysis and sequencing of the isolated BAC clone RPCIB753A07289. The gene consists of 13 exons spanning a genomic region of approximately 250 kb. Analysing possible gene function, database search using the translated gene product revealed homology to 3 hypothetical human proteins of yet unknown function sharing a striking homology of 120 aa at the C-terminal end suggesting the existence of a shared domain. This homology seems to be evolutionary conserved down to *D. melanogaster* and *C. elegans*. Recently, mouse cDNA clones have been isolated which will serve to verify the physical localisation in the distal region of MMU2 and the mouse gene will be the basis to investigate monoallelic expression using a translocation mouse model. This work was supported by a grant of the DHGP.



**Molecular characterization of a Rieger syndrome nonsense mutation in PITX2.** I. Saadi<sup>1</sup>, A. Kuburas<sup>2</sup>, A.F. Russo<sup>1,2</sup>. 1) Genetics Program; 2) Department of Physiology and Biophysics, University of Iowa, Iowa City, IA.

Rieger syndrome is an autosomal-dominant developmental disorder characterized by ocular anterior chamber anomalies, dental hypoplasia, mild craniofacial dysmorphism, and umbilical stump formation in humans. Mutations in the PITX2 bicoid-type homeodomain protein have been found in Rieger syndrome patients. The C-terminal tail of PITX2 has been shown to contain a DNA binding inhibitory domain and to be involved in protein-protein interaction with Pit-1. Here we report the characterization of a nonsense mutation (W133Stop) originally described by Semina *et al.* (1996) that truncated the C-terminal 139 amino acids of PITX2. We have performed electrophoretic mobility shift assays (EMSAs) using the W133Stop truncated protein to show greater binding than the wildtype protein to the bicoid site (TAATCC). Consistent with the greater DNA binding, the truncated protein also showed greater activation of the *Prolactin* and *Gad1* promoter-reporter constructs in CHO (Chinese hamster ovary) cells. However, co-transfection of wildtype PITX2 with W133Stop mutant resulted in suppression of the increased W133Stop activity in CHO cells. We are currently repeating these experiments in N2a (neuroblastoma) and LS8 (dental epithelium) cells. Preliminary results have shown that W133Stop poorly transactivated *Prolactin* and *Gad1* promoters in N2a cells, contrary to CHO cells. We are also examining the ability of W133Stop to dimerize with wildtype PITX2 using a yeast two-hybrid assay. Finally, we are testing the effect of W133Stop mutant following injection of mutant RNA into *Xenopus* embryos.

**The FMF Protein, Pyrin, Specifically Interacts with the SH3 Domain of the Cytoskeletal Protein PSTPIP1.** *N.G. Shoham, E. Mansfield, G. Wood, M. Centola, D.L. Kastner.* Inflammatory Biology Section, Rheumatic Disease Genetics and Genomics Branch, NIAMS,NIH, Bethesda, MD 20892.

Familial Mediterranean fever (FMF) is a recessive disorder characterized by episodes of fever and inflammation. Recently we identified the FMF gene (*MEFV*) by positional cloning; it is expressed at high levels in granulocytes and monocytes, and the protein product, pyrin, is associated with microtubules and actin filaments. Using the yeast two-hybrid system, we identified proteins that specifically interact with pyrin. One group of clones encodes the cytoskeletal protein, proline serine threonine phosphatase-interacting protein (PSTPIP1). PSTPIP1 is a CDC15p homolog that is known also to associate with the actin cytoskeleton and the Wiskott-Aldrich syndrome protein (WASP). We investigated the interaction domains of pyrin and PSTPIP1 as well as the influence of FMF-associated mutations on the interactions. Deletion and domain interaction analysis showed that the B-box and coiled-coil domains of pyrin are involved in the binding of pyrin to PSTPIP1. Fluorescence microscopy confirmed the binding by colocalization of full length pyrin or the B-box and coiled-coil domain with PSTPIP1 in transfected HeLa cells. Moreover, disease-associated mutations in pyrin altered the binding in the yeast two-hybrid system and changed the localization of PSTPIP1 in transfected cells. In the yeast two-hybrid system, analysis of PSTPIP1 interaction domains revealed that deletion of the SH3 domain completely abolished the binding to pyrin. Substitution of tyrosine 367 (found in the SH3 domain) to aspartate resulted in a loss of pyrin binding. These results suggest that the PSTPIP1 and pyrin interaction may be regulated by tyrosine phosphorylation of the PSTPIP1 SH3 domain, and that pyrin may regulate inflammatory responses at the level of cytoskeletal organization. FMF-associated mutations in pyrin appear to interfere with this interaction.

**Relative Rates of Disease Causing Missense Substitution are Predicted by the Level of Evolutionary**

**Conservation in *F9*.** W.A. Scaringe<sup>1</sup>, E.C. Thorland<sup>2</sup>, R.K. Pruthi<sup>2</sup>, D. Ricke<sup>2</sup>, K.A. Hill<sup>1</sup>, S.S. Sommer<sup>1</sup>. 1) Department of Molecular Genetics, City of Hope National Medical Center and Beckman Research Institute, Duarte, California, USA; 2) Department of Biochemistry, Mayo Clinic/Foundation, Rochester, Minnesota, USA.

The *in-vivo* effects of a missense substitution are typically variable even when truncating mutations to the gene are known to be devastating. The degree to which an amino acid is conserved through evolution has been demonstrated to be a useful indicator of its functional importance and may be viewed as the most direct measure of the *in-vivo* functional importance of the amino acid to reproductive fitness. The degree of cross species conservation was analyzed in detail in the factor IX gene family (*F9*, MIM No. 306900) and correlated with the spectrum of *F9* missense mutations observed in patients with hemophilia B which is caused with varying severity by different *F9* mutations. Members of the factor IX gene family were sequenced in non-mammalian vertebrates to supplement the existing database. By comparing more than 500 different missense changes known to cause hemophilia B in humans with the sequence variation among wildtype members of the factor IX gene family, the amino acids in the protein were classified into five levels of evolutionary conservation. The frequency of ascertained hemophilia B causing missense mutation was found to increase with the degree of evolutionary conservation. In addition, the observed disease causing variants were biased towards chemically dissimilar amino acids. These results further validate the utility of evolutionary conservation for predicting the impact of missense mutations and provides an approach for quantifying the likelihood that a missense change in a given candidate gene will be of functional significance. By helping to predict the likelihood that a missense change will be deleterious or neutral, evolutionary conservation analysis may be a cost-effective way to allocate resources for candidate gene analyses of complex disease.

**Expression of DAZAP1, a protein that interacts with the fertility factors DAZL and DAZ.** *Y. Vera*<sup>1</sup>, *T. Dai*<sup>1</sup>, *E. Salido*<sup>3</sup>, *T. Guo*<sup>2</sup>, *A. Sinha Hikim*<sup>2</sup>, *P. Yen*<sup>1</sup>. 1) Pediatrics, Harbor-UCLA Medical Center, Torrance, CA; 2) Medicine, Harbor-UCLA Medical Center, Torrance, CA; 3) Pathology, University of La Laguna, Spain.

DAZAP1 (DAZ Associated Protein 1) was originally identified through its interaction with two closely-related germ-cell specific proteins, DAZL and DAZ, that are required for germ cell development. DAZAP1 is a 407-amino-acid protein containing two RNA-binding domains and a proline-rich C-terminal portion. It is expressed most abundantly in testes. Its amino acid sequence is 83% identical to that of the *Xenopus* Prp protein which binds to the localization element in the 3' UTR of *Xenopus Vgl* mRNA. To elucidate the function of DAZAP1, we studied the expression and subcellular localization of DAZAP1 during spermatogenesis. RNA *in situ* hybridization showed that in adult testes *Dazap1* was expressed most abundantly in early premeiotic cells, from intermediate spermatogonia to preleptotene spermatocytes, and the level of expression decreased dramatically afterward. We generated two anti-DAZAP1 antibodies, one against a recombinant protein containing the C-terminal half of DAZAP1, and the other against an oligopeptide containing the last 19 amino acid residues of DAZAP1. Both antibodies bound to *in vitro* synthesized DAZAP1, and both detected a 46 kD protein in mouse testis extracts. Immunostaining of mouse testicular sections using affinity-purified antibodies showed a delay in the translation of *Dazap1* mRNA. DAZAP1 was first detected in both the cytoplasm and nuclei of mid-pachytene spermatocytes in stage VII tubules. In round spermatids DAZAP1 was detected mainly in nuclei, whereas in elongated spermatids DAZAP1 was present in the cytoplasm. DAZAP1 therefore appears to shuttle between the nucleus and the cytoplasm, and could be involved in RNA transport. In the cytoplasm, DAZAP1 was found not to be associated with polyribosomes.

**Endonuclease-Independent L1 Retrotransposition.** *T.A. Morrish, J.V. Moran.* Human Genetics, University of Michigan, Ann Arbor, MI.

Retrotransposition-competent L1s (RC-L1s) are 6.0 kb and contain a 5' untranslated region (UTR), two open reading frames (ORF1/ORF2), and a 3' UTR that ends in a poly (A) tail. ORF1 encodes an RNA binding protein, while ORF2 encodes a protein with endonuclease (EN) and reverse transcriptase (RT) activities. L1 likely mobilizes by target-site primed reverse transcription (TPRT). During TPRT, L1 EN cleaves the target sequence 5'-TTTT/A-3', liberating a 3' hydroxyl, which serves as a primer for reverse transcription of L1 RNA by the L1 RT. How the resultant cDNA joins to genomic DNA remains unknown. To ask if this process involves DNA repair proteins, L1 retrotransposition was assayed in a Chinese Hamster Ovary (CHO) cell line defective in non-homologous end joining (*xr-1*), and the corresponding wild-type parental cell line (4364a). As expected, RC-L1s readily retrotranspose in 4364a cells, and missense mutations in either the EN or RT domain reduced retrotransposition by 2-3 orders of magnitude. Remarkably, EN-independent (but RT-dependent) retrotransposition occurs in *xr-1* cells near wild-type levels. Comparison of insertion events in *xr-1* cells reveals that RC-L1s and EN-deficient L1s retrotranspose by different mechanisms. Five insertions derived from an RC-L1 have the predicted structure (i.e., they are 5' truncated, end in a poly (A) tail, integrate into a consensus L1 EN cleavage site, and generally are flanked by target site duplications (tsds)). By contrast, eight insertions derived from EN-deficient L1s have unusual structures; six are 3' truncated, none insert into an EN cleavage site, and all lack tsds. Detailed analysis of three EN-deficient integration events provides evidence that L1 can integrate at existing breaks in chromosomal DNA. Interestingly, in one instance 55bp of nucleolin RNA was co-retrotransposed with L1 RNA, suggesting that L1 intermediates may contain at least two mRNAs. In summary, our data suggest that pre-existing nicks in chromosomal DNA can function as primers to initiate cDNA synthesis of L1 RNA in an endonuclease-independent manner. This proposed mechanism curiously is analogous to that of telomerase, and provides an *in vivo* example of RNA mediated DNA repair.

**In Search of L1 Retrotransposition Intermediates.** *D.A. Kulpa, J.N. Athanikar, J.V. Moran.* Human Genetics, University of Michigan, Ann Arbor, MI.

LINES (L1s) are present in 850,000 copies in the human genome, comprising »21% of DNA. The majority of L1s cannot retrotranspose because they are 5' truncated or mutated; however, approximately 60 remain retrotransposition-competent (RC-L1s). RC-L1s are 6kb in length, and contain a 5' untranslated region (UTR), two open reading frames (ORF1 and ORF2), and a 3' UTR with a polyadenylic acid tail. ORF1 encodes an RNA binding protein (ORF1p), while ORF2 encodes a protein with endonuclease (EN) and reverse transcriptase (RT) activities (ORF2p). L1 retrotransposition requires the transcription of L1 RNA, its transport to the cytoplasm, and subsequent translation of its two open reading frames. Recent genetic experiments indicate the L1-encoded proteins preferentially bind to the transcript from which they were translated (i.e., they demonstrate a *cis*-preference) to form ribonucleoprotein (RNP) particles, which are proposed intermediates in the retrotransposition pathway. We currently are exploiting the *cis*-preference mechanism by selectively tagging L1s in an effort to test whether RNPs are indeed retrotransposition intermediates. Here, we demonstrate that L1s containing an epitope tag at the carboxyl terminus of ORF1p remain retrotransposition competent, and that the epitope tagged protein as well as its RNA localizes to cytoplasmic RNP complexes. Missense mutations in ORF1p, which block L1 retrotransposition, also block its ability to localize to RNPs. By contrast, a missense mutation in the RT domain of the ORF2p, which abolishes retrotransposition, does not affect localization of ORF1p. Together, our results demonstrate that ORF1p plays an important role in RNP formation, and lend support to the hypothesis that RNPs are L1 retrotransposition intermediates.

**Deletion of Azoospermia Factor b(AZFb) in infertile men caused by non-homologous recombination between Alu and LINE-1 elements.** *E. MORO<sup>1</sup>, A. FERLIN<sup>1</sup>, A. ROSSI<sup>1</sup>, P. MARIN<sup>1</sup>, B. DALLAPICCOLA<sup>2</sup>, C. FORESTA<sup>1</sup>.* 1) Medical & Surgical Sciences, University of PADOVA, ITALY; 2) Department of Medical Genetics, University of ROME.

Microdeletions of the Yq are the most common mutation observed in infertile males, where they affect one or more azoospermia factors (AZFa, b and c). Understanding of the AZF structure and gene content and mapping of the deletion breakpoints in infertile men are still incomplete. About fifty percent of the Y chromosome consists of repetitive elements, such as Alu, DNA transposons and retroviral sequences so that illegitimate recombination mechanisms have been evoked as possible causes of AZF deletions. Recently, two homologous retroviral sequences of the HERV family have been identified in the AZFa region and it has been suggested that their recombination is causing most of AZFa deletions. AZFb region has been less analyzed due to its complex structure. It has been proposed that RBMY-1 gene family represent the candidate gene, being expressed only in the testis and specifically in germ cells. However, deletions within AZFb that do not remove RBMY-1 have been described and no RBMY-1 point mutation have been identified yet. Patients with AZFb deletion not involving RBMY-1 seem to have indistinguishable proximal and distal breakpoints, suggesting a common responsible mechanism. We have reviewed the AZFb map of BAC clones and shown that it contains different inverted repeats. Using sequence-tagged sites (STS) analysis approach we have identified common breakpoints in four unrelated infertile individuals with deletion within AZFb. By means of electronic analysis we found a block containing an Alu element in the distal breakpoint, and another block containing a cluster of LINE-1 elements in the proximal breakpoint region. These findings sustain an illegitimate recombination between Alu and LINE1 elements as a cause of the AZFb deletion. The four male infertile patients were not deleted for the active copy of RBMY-1 gene family. It can be hypothesized that the deletion may have deregulated RBMY-1 expression or alternatively other genes mapped in the AZFb may have a role in the spermatogenesis.

**SVA elements are novel active human retrotransposons that cause disease.** *E.M. Ostertag, Y. Zhang, H.H. Kazazian, Jr.* Dept. of Genetics, University of Pennsylvania, Philadelphia, PA.

SVA elements were previously described as composite retroposons, consisting of a SINE-R region, a VNTR region, and an Alu region. Our database analysis of SVA elements suggests that they are non-autonomous retrotransposons, which are mobilized by the retrotransposition machinery of the L1 retrotransposon. The following data support our hypothesis: 1) SVA elements end in an AATAAA poly-A signal, followed immediately by an A-rich tail. This structure is similar to the L1 3' end and may be required for the integration process. 2) SVA elements have target site duplications (TSDs) identical to L1 TSDs, suggesting that they are created by the L1 endonuclease. 3) SVA elements are occasionally 5' truncated, a phenomenon common during L1 retrotransposition. In fact, we propose that SINE-R retroposons are simply 5' truncated SVA insertions. 4) SVA elements are able to transduce 3' flanking sequence, an indirect consequence of using the L1 retrotransposition process. 5) SVA elements can 5' invert during the retrotransposition process, another phenomenon common during L1 retrotransposition. The report of an evolutionarily recent insertion of a SVA element into the fukutin gene suggested that SVA elements may be currently active. We have now identified other recent SVA insertions, an insertion into the Btk gene (previously described as a SINE-R insertion), and an SVA-mediated transduction (see #4 above) inserted into the  $\alpha$ -spectrin gene (previously described as a novel SINE insertion). We find many copies of SVA elements with greater than 99% identity to the SVA insertions, supporting recent activity. Additionally, we are able to identify the empty site of one SVA insertion precursor, suggesting that some SVAs are polymorphic in the population and therefore recently active. Lastly, we demonstrate in a cultured cell assay that SVA elements are active non-autonomous retrotransposons and that they are able to retrotranspose using the L1 retrotransposition machinery.



**Novel HERV-K genes (ERV3 and ERV4) were mapped to autoimmune disease loci on chromosome 3. J.**

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Human endogenous retroviruses (HERVs) are LTR-containing retroelement, which are widely spread throughout the human genome. The vast majority of HERV sequences are dysfunctional due to the accumulation of multiple nonsense mutations. HERV-K genes, one of the HERV family members, are estimated to reside as about 30 to 50 copies in the haploid human genome. Recently HERV-K18/IDDMK12,22 has been shown to code for a superantigen and to possibly implicate in the pathogenesis of autoimmune diabetes. To explore the pathological role in human disorders, we tried to identify transcriptionally active other members of HERV-K genes. Preceding the isolation, expression status was examined by RT-PCR and sequence using mRNAs from various tissues. In addition to detection of HERV-K18/IDDMK12,22 in peripheral leukocytes, two novel HERV-K genes (ERV4 and ERV5) were detected in placenta, lung, testis, and peripheral leukocytes, then subsequently isolated from a BAC library using oligonucleotide-probes and characterized. Although their expression could not be confirmed in normal tissues tested by northern blot analysis, substantial promoter activity of their 5' LTRs was demonstrated in luciferase assays using teratocarcinoma cell lines. Thus, they seem to have a potential to be actively transcribed. These results indicated that subtle base changes in LTR sequences could be responsible for gene regulation such as tissue specificity of HERV-K expression. To evaluate the involvement in any human diseases, G3 RH panel using markers designed from flanking sequences on BAC clones were performed. ERV4 and ERV5 were mapped to chromosomal regions 3q21-q25.2 and 3cen-q13, respectively. Recent progress of human genome project helped us to know the precise gene location on 327kb (Hs3\_22567) and 898kb (Hs3\_22660) clone contig. In these regions several susceptibility loci for autoimmune diseases (type 1 diabetes, MS, SLE, and RA) were reported by genome-wide linkage studies. ERV4 and ERV5 could play a role in pathogenesis of these diseases.

**The FMR1 Message Facilitates Translation by Internal Ribosome Entry.** *P. Chiang, L. Carpenter, F. Tassone, P.J. Hagerman.* Department of Biological Chemistry, School of Medicine, University of California, Davis, California.

Fragile X syndrome, the leading heritable form of mental impairment, is nearly always caused by large expansions of a CGG trinucleotide repeat element in the promoter region of the FMR1 gene, with consequent absence of FMR1 protein (FMRP). For most individuals with fragile X syndrome, absence of FMRP is due to transcriptional silencing. However, for some individuals, no protein is produced despite continued activity of the FMR1 gene. This observation suggests that translation of the FMR1 message is impaired, presumably due to the expanded CGG element in the 5' untranslated region (UTR) of the FMR1 message, although no causal relationship between the size of the CGG element and efficiency of translation has been established. To examine the properties of the FMR1 5'UTR, nested deletions were generated within a normal FMR1 5'UTR, for both monocistronic and dicistronic (luciferase) reporter constructs. Surprisingly, in transient transfection experiments performed with cell lines of both neural and lymphoid origin, a ~30 nucleotide segment in the 5'UTR was found to promote translation by internal ribosome entry. Additional controls indicate that the enhanced activity of the downstream reporter is not due to readthrough from the upstream cistron, nor is it due to translation of cryptic monocistronic transcripts. The functional significance of this internal ribosome entry site (IRES) is not known at present, although it may act as a target for context-dependent regulation of the translation of FMR1 mRNA.

**Pharmacological Reactivation of the *FMRI* Gene of the Fragile X Syndrome.** *G. Neri*<sup>1</sup>, *M.G. Pomponi*<sup>1</sup>, *R. Pietrobono*<sup>1</sup>, *P. Chiurazzi*<sup>2</sup>. 1) Institute of Medical Genetics, Catholic University, Rome, Italy; 2) Department of Pediatrics, University Hospital, Messina, Italy.

Fragile X syndrome is the leading cause of heritable mental retardation, with a prevalence in the general population of 1:4000 males. Most fragile X patients have an amplified CGG repeat in the promoter of the *FMRI* gene, which becomes abnormally methylated and inactive. We demonstrated that reactivation of the fully mutated gene can be achieved by treating patients' lymphoblasts with the demethylating drug 5-azadeoxycytidine (*Hum Mol Genet* 7: 109-113, 1998) and, although at very low levels, also with the histone deacetylase inhibitors 4-phenylbutyrate or butyrate, that should cause histone hyperacetylation. However, when 4-phenylbutyrate or butyrate were used with 5-azadeoxycytidine, a marked synergistic effect was observed (*Hum Mol Genet* 8:2317-2323, 1999), thus confirming that DNA hypermethylation and histone deacetylation are sequential steps leading to the transcriptional silencing of the fully mutated *FMRI* gene. We now report on the correlation between reactivation of the gene following treatment with 5-azadeoxycytidine and actual demethylation of the promoter. By bisulfite sequencing we determined that the treatment leads to a nearly complete demethylation of the promoter. On the other hand, Southern analysis after digestion of the DNA with methylation sensitive enzymes suggests that demethylation of the CGG sequence is probably more limited. This could explain why in cells with larger CGG expansions the attainable level of reactivation is less than in cells with shorter expansions. We also intend to verify whether treatments with histone deacetylase inhibitors determine a significant level of histone hyperacetylation and/or DNA demethylation of the *FMRI* promoter. Supported by grants from the FRAXA Foundation and from Sigma-Tau.

**Cell type specific effect of CTG expanded repeats on the expression of a reporter gene.** *C. Montanez<sup>1</sup>, B. Cisneros<sup>1</sup>, R. Korneluk<sup>2</sup>, F. Depardon<sup>1</sup>.* 1) Genetics and Molecular Biology Department, Cinvestav, IPN. Av. I.P.N. 2508. C. P. 07360. Mexico, D.F. Mexico; 2) Solange Gauthier Karsh Molecular Genetics Laboratory, Children's Hospital of Eastern Ontario, Ottawa, Ontario K1H 8L1, Canada.

Myotonic dystrophy (DM) results from the expansion of an unstable (CTG)<sub>n</sub> trinucleotide repeat within the 3'-untranslated region (3'-UTR) of the DMPK gene (human chromosome 19q13.3). The mechanism by which the CTG repeat expansion mutation leads to the clinical phenotype of DM is unknown. In order to understand how the CTG expanded repeats located at the 3' untranslated region exerts an effect on gene expression, we analyzed the expression of the reporter gene CAT containing at the 3' untranslated region 0, 5, 45 and 90 CTG repeats in L6 rat myoblasts and SH-SY5Y human neuroblastoma cell lines. For this, RT-PCR, Western blots, in vitro translation and Cat activity determination assays were performed. Our results show that the expression of the CAT reporter gene at the transcription level was similar in all constructs and cell lines analyzed. Most of the CAT mRNAs encoded by all plasmids used were detected in the cytoplasmic fraction. Interestingly, CAT activity and synthesis was dramatically reduced in L6 cells transfected with the CAT vectors carrying 45 and 90 CTG repeats. In opposition to this, the CAT expression was found to be unaltered in SH-SY5Y human neuroblastoma cells. These results show that the CTG expanded repeats inhibitory effect on CAT expression, is cell type dependent.

**Functional studies on the Human 5'-CGG-3'-Binding Protein CGGBP1 (p20).** *F. Naumann, H. Müller-Hartmann, H. Deissler, W. Doerfler.* Institute of Genetics, University of Cologne, Cologne, Germany.

From the nuclei of human HeLa cells, we have isolated a 20 kDa protein which binds specifically to 5'-CGG-3' repeats, either in synthetic oligodeoxyribonucleotides or in the 5'-upstream region of the FMR1 gene on human chromosome Xq27.3. The loss of the FMR1 (fragile X mental retardation) gene function has been implicated in the causation of the fragile X syndrome which is associated with an unstable trinucleotide repeat. The specific binding of CGGBP1 is compromised when the repeat sequence is methylated. The amino acid sequence of three peptides in the CGGBP1 has been determined, and an EST clone with the matching nucleotide sequence has been available. Screening of the protein data bases has not revealed a related sequence. The genomic nucleotide sequence of the gene for the human CGGBP1 has also been determined, and the gene structure has been analysed. The human gene maps to the paracentric region on the short arm of chromosome 3 as shown by FISH. The human CGGBP1 is ubiquitously expressed. It can be alternatively spliced and polyadenylated. The CGGBP1 gene is highly conserved among mammals and birds. Cloning of the murine gene revealed a 98% identity in the amino acid sequence. To investigate the function of the murine CGGBP1 *in vivo*, we have initiated a conditional gene targeting experiment in C57BL/6 mice and have completed appropriate constructs. In cotransfection experiments, the activity of the FMR1 promoter in luciferase constructs is reduced by the overexpression of the CGGBP1. Upon transfection of the CGGBP1 cDNA construct into HeLa cells, transcription of the endogenous FMR1 gene is also decreased. In a quest for additional CGGBP1 targets, we have initiated microarray experiments using RNA from human HeLa cells that had been transfected with a HCMV promoter-CGGBP1 construct or with control constructs. Several differentially expressed candidate genes in cells overexpressing CGGBP1 are currently investigated. How many cellular genes are affected in their transcriptional activity by the presence of the CGGBP1? [This research was supported by the Center for Molecular Medicine Koeln - TV13].

**Alpha1A voltage-dependent calcium channel gene with expanded CAG repeats exerts caspase dependent cytotoxicity in HEK cell lines.** *K. Owada<sup>1</sup>, T. Kubodera<sup>1</sup>, K. Ishikawa<sup>1</sup>, M. Li<sup>1</sup>, T. Tsunemi<sup>1</sup>, S. Toru<sup>1</sup>, T. Tanabe<sup>2</sup>, H. Mizusawa<sup>1</sup>.* 1) Department of Neurology and Neuroscience, Graduate School, Tokyo medical and Dental University, Japan, MD; 2) Pharmacology and Neurobiology, Graduate School, Tokyo medical and Dental University, Japan, MD.

Spinocerebellar ataxia type 6 (SCA6) is caused by small CAG repeat expansion which code polyglutamine in the alpha1A voltage-dependent calcium channel gene (CACNA1A). Although the slowly progressive degeneration and loss of Purkinje cells is the main feature in SCA 6, the mechanisms that CACNA1A with CAG-repeat expansion leads to neurodegeneration have not been clarified. However, a line of evidence suggests that the Purkinje cell neurodegeneration may be associated both with functional alteration of calcium channel and expanded polyglutamine itself that causes microaggregation of mutated protein as seen in other polyglutamine diseases. To clarify the mechanism of cell death in aspect of polyglutamine toxicity, we generated HEK cell lines which stably express human CACNA1A either with normal 13 or expanded 28 CAG-repeats. Although the repeat expansion did not show cell toxicity under normal condition, serum deprivation stress markedly increased TUNEL positive apoptotic cells in the CAG-repeat expanded cell lines. In addition, caspase inhibitor suppressed the cell death induced by serum deprivation. These results suggest that CAG-repeat expansion of CACNA1A exerts caspase depending cytotoxicity in HEK cell lines.

**A comparative study of SCA7 cell models and human brain: recruitment of proteins into inclusions and activation of caspase-3** è. C. Zander<sup>1,5</sup>, J. Takahashi<sup>2</sup>, KH. Hachimi<sup>4</sup>, H. Fujigasaki<sup>1</sup>, C. Duyckaerts<sup>2,4</sup>, A. Brice<sup>1,3</sup>.  
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Spinocerebellar ataxia type 7 (SCA7) is one of the nine known polyglutamine diseases. SCA7 is an autosomal dominant neurodegenerative disorder, characterized by visual loss and cerebellar ataxia. Pathologically, there is a progressive pigmentary macular degeneration and predominant neuronal loss in the cerebellar cortex, brain stem and inferior olivary complex. The molecular pathways leading to selective neuronal death in SCA7 are poorly understood.

We have established a cell culture system based on transiently expressed tagged variants of SCA7 in two human cell lines: HEK293 (embryonic kidney) and SH-SY5Y (neuroblastoma), that mimic several of the major characteristics of SCA7. The cells readily formed fibrillar aggregates with an amyloid-like structure that recruited other proteins. In this study we have characterized these cells and compared the inclusions with those in human SCA7 brain tissue. This will allow comparison with SCA7 mice models and other polyglutamine disorders. There were consistent indirect signs of an ongoing abnormal protein folding, including the recruitment of proteasome subunits and heat shock proteins. Occasionally, there was also sequestration of transcription factors in the brain (CBP and mSin3a) and in a small subset of the aggregates in the SH-SY5Y cells (CBP and p53). A possible role of caspase-3 activation in SCA7 pathogenesis was evoked in our study by its recruitment into the aggregates/inclusions. Finally, on the ultrastructural level there were signs of catabolic dysfunction and nuclear indentations as major cell stress response in the transfected cells.

**Antisense expression at the human *XIST* locus does not prevent localization of the *XIST* RNA.** *J.C. Chow*<sup>1</sup>, *L. Hall*<sup>2</sup>, *J.B. Lawrence*<sup>2</sup>, *C.J. Brown*<sup>1</sup>. 1) Dept Medical Genetics, Univ British Columbia, Vancouver, Canada; 2) Univ Massachusetts Medical Centre, Worcester, MA.

Mammals employ X-inactivation to transcriptionally silence most genes on one of the two X's in females. This process requires *XIST*, an untranslated RNA that is expressed exclusively from the inactive X. The localization of the transcript to the inactive X in interphase nuclei suggests that the RNA may have a structural role. In mouse, an antisense transcript that may regulate *Xist* expression initiates 15 kb downstream of *Xist* and completely overlaps the *Xist* locus. Targeted deletions of the antisense promoter indicate that it is involved in both imprinted and random X-inactivation. We have identified antisense transcription at the human *XIST* locus that also appears to be specific to undifferentiated cell types, being observed in two different embryonal carcinoma lines, N-Tera2D1 and Hs 444, but not in somatic cells. Unlike the murine antisense transcript, the human transcript does not completely overlap the *XIST* gene and is not correlated with destabilisation of the sense transcript. While no expression of the human antisense transcript is seen in somatic cells, transient expression can be induced by demethylation, suggesting that, as with the *XIST* gene, methylation is important in silencing of the locus.

To examine the role of the antisense transcript in the localisation of *XIST* to the X chromosome, we have transfected a PAC containing at least 20 kb flanking the transcriptional start sites of both the sense and antisense transcripts into human somatic cell lines. We have identified a transfectant that expresses both sense and antisense transcripts, and despite substantial antisense expression, RNA-FISH indicates that the sense *XIST* transcript still forms a large signal that localises to surrounding chromosomal material. Therefore, antisense expression does not appear to significantly decrease sense *XIST* levels or preclude localization in somatic cells. Our results favor models of antisense action involving developmental, stage-specific factor binding over models involving antisense RNA destabilisation of the *XIST* RNA.



**The ICF syndrome as a model for studying inactive X silencing: methylation versus acetylation.** *R.S. Hansen<sup>1</sup>, S.M. Gartler<sup>1,2</sup>*. 1) Medicine/Medical Genetics, University of Washington, Seattle, WA; 2) Genetics, University of Washington, Seattle, WA.

The inactive X is silenced by a multiplicity of factors whose interrelationships have yet to be fully worked out. To understand the relative roles of such factors, one approach is to study the inactivation system when it is at least partially destabilized. The rodent-human somatic cell hybrid with a human inactive X is one such model system that has been used to study inactive X silencing for the last 25 years. Destabilization of X inactivation in this system appears to result, at least in part, from an alteration of the XIST RNA sex chromatin body. The ICF immunodeficiency syndrome provides a new cellular model for studying the human inactive X silencing complex. We and others have shown that ICF syndrome can result from mutations in the *DNMT3B* DNA methyltransferase gene. This methyltransferase targets various forms of heterochromatin including the inactive X, and we have shown that all gene-associated CpG islands on the inactive X appear to be hypomethylated. These regions are commonly found to be mildly advanced in their time of replication and retain normal silencing. In cases of markedly advanced replication, however, escape from inactivation is found. Sex chromatin structure, including XIST RNA association, appears normal in these cells. We now report new examples of escape from inactivation in ICF as well as new examples of advanced replication timing for single copy and repeated sequences. In addition, our recent studies of histone acetylation in ICF female cells provide an interesting challenge to the view that methylation in heterochromatic regions brings about deacetylation by attracting deacetylases. We find that even though the CpG islands of the inactive X in ICF female cells are extensively hypomethylated, the histones are hypoacetylated normally at the cytological level. These data also call into question the need for hyperacetylation in the regions of genes that escape inactivation.

**XIST Expression from the Active X: A Hybrid Model System for Studying the Role of XIST independent of Gene Silencing.** *E.S.H. Kwok, K. Harris, C.J. Brown.* Medical Genetics, Univ British Columbia, Vancouver, BC., Canada.

X-inactivation results in the transcriptional silencing of one of the two X chromosomes present in mammalian female cells. XIST expression from the inactive X (Xi), and subsequent coating of the Xi by the XIST RNA, marks the initial major observable characteristic of X-inactivation. The Xi then begins to exhibit features of heterochromatin such as hypermethylation, histone deacetylation, late replication, and the recruitment of core histone variants. Ultimately most X-linked genes on the Xi become silenced, but the exact mechanistic events between XIST expression and the final heterochromatic state of the Xi remain unclear.

To better understand the relationship between XIST, heterochromatin, and silenced gene expression, we have developed a model system to examine which features of heterochromatin can be induced by XIST independent of gene silencing. Rodent/human hybrids containing active human and mouse Xs were demethylated to reactivate XIST/Xist expression. Previous work showed that while human XIST fails to localize to the human X, the mouse Xist localizes normally. In both cases no silencing of X-linked genes was observed. To verify this hybrid cell system as a model for studying the effects of XIST/Xist independent of inactivation, we characterized the methylation and expression status of 8 mouse/human genes and found them to be unchanged. This confirmed the lack of X-linked gene silencing despite the presence of XIST/Xist expression.

The first feature of the Xi we are investigating is the colocalization of the histone variant macroH2A1.2 (mH2A), since the loss of Xist expression has been shown to result in mH2A delocalization. We have made a macroH2A-GFP construct to be expressed in the described model system to study whether XIST expression is sufficient to recruit mH2A to the X chromosome, or if mH2A localization requires the silenced chromatin structure of the Xi. How mH2A and other features act in the presence of XIST but in the absence of a silenced Xi state will help define their roles in X-inactivation.

**DNMT1 is not required for maintenance of *XIST* repression in human cancer cells.** *L.R. Vasques, M. Soukoyan, L.V. Pereira.* Dept. Biologia, Instituto de Biociencias, Universidade de São Paulo, SP, Brazil.

In mammals, dosage compensation of X-linked gene products between XY males and XX females is achieved by transcriptional inactivation of one X chromosome in females. The *XIST/Xist* gene is expressed exclusively from the inactive X ( $X_i$ ), and seems to trigger initiation of X chromosome inactivation (XCI) in cis. Repression of *XIST/Xist* on the active X ( $X_a$ ) chromosome upon differentiation has been associated with methylation of its 5' region. This region is hypermethylated on the  $X_a$ , where *XIST/Xist* gene is repressed, and hypomethylated in the *XIST/Xist* expressing  $X_i$ . To date, four different mammalian DNA (cytosine-5) methyltransferase (MTases) have been identified: Dnmt1, Dnmt2, Dnmt3a and Dnmt3b. Dnmt1 is constitutively expressed, has higher activity in hemi-methylated DNA, and thus has been recognized as a maintenance MTase. The role of Dnmt1 in the process of DNA methylation and XCI has been extensively studied in mice, where Dnmt1 activity has been shown to be causally involved in maintenance of global DNA methylation and particularly in the transcriptional repression of the *Xist* gene.

In humans, disruption of the *DNMT1* gene by homologous recombination in the human carcinoma cell line HCT116 (46, XY) led to a reduction of only 20% of overall DNA methylation. These data indicated that human DNMT1 is involved in DNA methylation differently than its murine counterpart, and prompted us to investigate the role of DNMT1 in the process of maintenance of *XIST* gene repression on the  $X_a$  in that human cell line. We show that methylation of the *XIST* promoter region and *XIST* transcriptional repression is sustained in DNMT1 deficient cells. Treatment of these cells with 5-aza-2-deoxycytidine leads to demethylation of the *XIST* promoter region and *XIST* gene expression. Our results in the human cell line HCT116 indicate that, although methylation is required for maintenance of *XIST* repression in the X chromosome, DNMT1 is not the MTase involved in this process in humans. Moreover, they suggest that the role of that enzyme in human XCI may not be extrapolated from data obtained in mice.

**First do no harm: an ethical counseling dilemma.** *D.L. Thull, S.M. O'Neill.* Cancer Genetics Program, UPCI/Magee Womens Hosp, Pittsburgh, PA.

An almost 17 year old young woman was referred by her PCP for genetic counseling and possible testing for *BRCA1* and *BRCA2*. The proband's maternal history was highly suspicious of the Hereditary Breast-Ovarian Cancer (HBOC) syndrome. Her mother was diagnosed with breast cancer at age 25 and died at age 34. Many maternal relatives were noted to have early onset breast or ovarian cancer. The proband is very concerned about her breast cancer risk, performs frequent self-breast exams and has already had a clinical breast exam, mammography and ultrasound screening. In obtaining the family history, first from the proband's stepmother and then from a maternal aunt, a history of Huntington Disease (HD) was elicited. Three of the proband's maternal uncles are affected with HD as was her maternal grandfather. The proband is somewhat aware of the HD in the family. Her stepmother had known of only one affected uncle and the grandfather. The referral raised the ethical issues of patient autonomy and nonmaleficence with respect to cancer predisposition testing in an adolescent. Furthermore, the presence of HD in the family raised the question of the legal responsibility of the genetic counselor to discuss HD versus the ethical concern regarding the potential to overwhelm and cause excessive mental burden to the young proband.

**Prenatal counseling in Friedreichs ataxia. An unexpected and unwelcome outcome.** *H. Cox, J. Macpherson.*  
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Hampshire, United Kingdom.

Friedreichs ataxia is the most common autosomal recessive hereditary ataxia. Classically, the onset of symptoms occurs before 20 years of age. In 1996 the gene for Friedreichs ataxia was discovered. The majority of affected individuals are homozygous for an unstable expansion of GAA repeats in the first intron of the frataxin gene on chromosome 9 (normal= <20 repeats). The availability of a diagnostic test has led to the recognition of a broader phenotype with a later onset of symptoms. Carrier frequency in the general population is 1/110. The risk to offspring of both affected patients and healthy carriers is therefore low.

A healthy 30 year old woman whose 28 year old sister suffers from Friedreichs ataxia presented to us in the 15th week of her first pregnancy. Her sister's diagnosis was confirmed and she was counseled that the risk to her offspring was low (1/660). She and her partner requested carrier testing for further reassurance. The patient was found to have expansions in both alleles (estimated sizes of 160 and 1200 repeats). Both of her partner's repeat sizes fell within the normal range. Our patient's sister had symptoms from 18 years of age. Diagnosis was at 25 years of age and she remains fully ambulant. Expansion sizes were estimated to be 300 and 1200. Meiotic instability of the smaller expansion would account for the difference in allele sizes and clinical presentation between the 2 sisters. Our patient was shocked by this molecular diagnosis, declined neurological examination and denies any neurological symptoms.

As genetic testing becomes possible for more recessive disorders, many patients request that genetic counseling be backed up by a test. Failure to counsel the consultand regarding her a priori risk of having Friedreichs ataxia resulted in an unexpected predictive test in pregnancy. This report is a timely reminder to genetic counsellors that carrier testing can be fraught with difficulties.

**Treatment of Multiple Sclerosis: Teratogenic Concerns and Other Aspects of Reproductive Counselling.** *A.D. Sadovnick, C. Guimond, E. Dwosh.* Medical Genetics, VHHSC-UBC, Univ British Columbia, Vancouver, B.C., Canada.

Multiple sclerosis (MS) is the most common neurological disorder affecting young adults during the ages when reproduction is a major consideration. Two-thirds of affected individuals are females. Genetic factors are known to influence MS susceptibility and evidence suggests that they also may impact on clinical course. Young couples, one of whom has MS, are increasingly approaching genetic clinics to request information on reproduction.

MS treatments fall into three categories: (1) symptom-specific (e.g. fatigue, bladder incontinence, optic neuritis, ataxia); (2) relapse/exacerbation management and (3) disease modifying (e.g. immunosuppressants believed to reduce the number of clinical relapses/exacerbations and lesions on MRI). Within each category, a variety of therapeutic agents are used singly or in combination. Thus, each individual's clinical course and treatment regime must be reviewed in detail as a critical component of reproductive counselling. This is true for all affected consultands, whether male or female, although some concerns will be specific to females.

The impact of commonly used therapeutic agents will be discussed with respect to fertility, teratogenicity, and breastfeeding. In addition to teratogenic concerns, other topics relevant to reproductive counselling for persons with MS will be outlined. These include (1) Effects of MS on Pregnancy (conception, pregnancy management, delivery, pregnancy outcome); (2) Effects of Pregnancy on MS (short-term and long-term concerns); (3) Contraception; (4) Recurrence Risks and (5) Psychosocial Issues.

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Program Nr: 1121

**Identification of familial breast cancer risk during prenatal genetic counseling.** *Y. Furman, D. Cutillo, D. Ramsey, A.E. Donnemfeld.* Genzyme Genetics, New York, NY and Philadelphia, PA.

Family history data regarding breast cancer, obtained at the time of prenatal genetic counseling, was tabulated during the months of March through May, 2001. 70 genetic counselors from 13 states obtained detailed family history information during all prenatal counseling sessions. The family history information was uniformly obtained and prepared in accordance with the recommendations for standardized human pedigree nomenclature (*Am J Hum Genet*, 56;745,1995). Information was entered immediately following the counseling session utilizing a lap-top clinical computer system to ensure accurate and complete data collection. During this 3-month interval, 7,551 patients were seen for prenatal genetic counseling. The mean maternal age of patients was 32 years (range 15-48). 791 patients (10.5%) were identified as having a family history of breast cancer. First degree relatives with breast cancer (mothers and sisters) of the expectant couple were identified on 392 occasions (5.2%). There were 233 maternal and 159 paternal first degree relatives identified. Second degree relatives (aunts and grandmothers) of the expectant couple were identified on 321 occasions (4.3%). There were 240 maternal and 81 paternal second degree relatives identified with breast cancer. There were 139 families with 2 affected relatives, 28 families with 3 affected relatives and 4 families with 4 affected relatives. 13 pregnant women and 2 expectant fathers had themselves been diagnosed with breast cancer. There were 7 males with breast cancer identified. The vast majority of patients who gain access to genetic counseling do so in the prenatal setting. Despite the fact that the primary indication for referral of prenatal patients involves a discussion of fetal risk for a genetic disorder, there is also an opportunity (and an obligation) to provide a thorough and comprehensive risk assessment for other genetic disorders identified during the counseling session, including breast cancer. Our experience is that these patients, although commonly anxious about their pregnancies, are receptive to a discussion of breast cancer risk if a significant family history is identified.

**Communication of Cancer Risk with Family Members and Evaluation of Duty to Inform.** *J. Choi<sup>1</sup>, C. Arbayo<sup>1</sup>, S. Sand<sup>1</sup>, P. Mantha<sup>1</sup>, K. Blazer<sup>1</sup>, R. Nedelcu<sup>1</sup>, M. Grant<sup>2</sup>, J. Weitzel<sup>1</sup>, D. MacDonald<sup>1</sup>.* 1) Dept Clinical Cancer Genetics; 2) Dept Nursing Research and Education, City of Hope (COH) Nat'l Medical Ctr, Duarte, CA.

More information is needed about the impact of the genetic cancer risk assessment (GCRA) process on risk perception, communication and healthcare in women concerned about hereditary breast or ovarian cancer. As part of this study, women with a personal or family history of breast or ovarian cancer were mailed follow-up questionnaires post-GCRA. We report here observations on risk communication and perceptions of duty to inform relatives of cancer risk, for the first 100 participants (51 subjects had breast cancer, 6 had ovarian cancer, and 43 were unaffected). Almost all participants discussed cancer risk with appropriate first-degree relatives after counseling. Ninety-two percent discussed risk with sisters. Among the 72% of women who discussed risk with brothers, 13/28 tested positive for a *BRCA* mutation and all informed their brothers regardless of whether the brothers had daughters, demonstrating integration of knowledge about cancer risk in these men's families. Six of the 8 women who discussed risk with more distant relatives had informative genetic testing results, perhaps showing that informative genetic testing motivated discussion with more distant relatives. Encouragingly, 71% reported that post-counseling they were better prepared to discuss risk with relatives because of a better understanding of risk and risk management. The most common barriers to discussing risk were: no contact with relatives (31%), relatives too far away (19%). Ninety-six percent of respondents felt that a duty to inform relatives about cancer risk exists. Interestingly, 18% felt that both they and the healthcare provider bear this responsibility. These preliminary findings suggest that GCRA influences risk communication positively and provide important data regarding beliefs about duty to inform that could help establish standard of care concerning genetic information. This research was supported by funds from the California Breast Cancer Research Program of the University of California, Grant Number 5BP-0051.



**The medical geneticists role in the multidisciplinary breast cancer pre-surgical treatment planning conference.**

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Historically, the Breast Cancer Conference has been composed of pathologists, surgeons and oncologists who gathered post-surgery to discuss pathology findings and treatment options. New imaging technologies and better treatment options have allowed the development of Pre-surgical Treatment Planning Conferences. For the last 6 years medical geneticists at our institution have been attending the weekly Conferences. As the important role of genetics in cancer diagnosis, prognosis and treatment became more apparent the geneticists have been called upon more. The cancer geneticists are now vital conference members as demonstrated in the following cases. A 53-year-old woman was presented regarding a planned lumpectomy. Prior to the conference a genetic counselor had telephoned the patient and learned of a paternal history of breast cancer and Ashkenazi Jewish ancestry. At the conference immediate predisposition gene testing was recommended, citing recent studies that favor less conservative surgery in the presence of BRCA1/2 mutations. Testing was positive for a BRCA1 mutation and the patient underwent mastectomy and is considering further prophylactic surgery. A 35-year-old woman with a palpable mass and no family cancer history was presented. Mammography suggested two lesions that appeared to be separated by 2-3 cm. Core biopsies of both lesions showed invasive cancer. Separate primaries could indicate an inherited cancer predisposition and mastectomy would be considered. If the two lesions were actually contiguous, more conservative surgery could be utilized. While additional imaging could address this question, a molecular approach could yield helpful information. A battery of molecular markers could be studied in both core biopsies. Concordance would suggest common origin; whereas, dissimilarity would suggest separate primaries. Tumor conferences at many medical centers have now become presurgical treatment planning conferences. Genetic counselors and medical geneticists can add knowledge and experience to these Conferences and contribute to overall patient care.

**Using Video Conferencing to Extend Genetic Cancer Risk Assessment Expertise to Community-Based Medical Centers.** *S. Sand*<sup>1</sup>, *R. Nedelcu*<sup>1</sup>, *I. Grady*<sup>2</sup>, *J. Congleton*<sup>3</sup>, *K. Blazer*<sup>1</sup>, *J. Choi*<sup>1</sup>, *D. MacDonald*<sup>1</sup>, *J. Weitzel*<sup>1</sup>. 1) Clin Cancer Genetics (CCG), City of Hope (COH) Natl Med Ctr, Duarte, CA; 2) North Valley Breast Clinic (NVBC), Redding, CA; 3) Good Samaritan Regional Medical Center, Phoenix, AZ.

Comprehensive genetic cancer risk assessment (GCRA) services are often limited to academic medical centers in large, urban communities. The COH *Cancer Screening & Prevention Program Network* (CSPPN) provides GCRA and conducts weekly CME-accredited, multi-disciplinary Working Group conferences to review all cases. The NVBC, staffed by a breast surgeon and nursing staff, provides GCRA since the nearest genetic counselor (GC) is 150 miles away. A CSPPN satellite in Phoenix, AZ, staffed by a board certified nurse/GC, provides GCRA with clinical and programmatic support from the CSPPN. **Objective:** To address geographic disparity in access to quality GCRA, we investigated the use of multipoint videoconferencing (VC) as a tool for collaboration between the CSPPN and two distant community medical centers. **Methods:** The COH CCG Technology Transfer Research project, supported by the California Research Program, was established to foster access to comprehensive GCRA in communities lacking such services. We installed VC units in the two community-based centers to provide consultation and case management through the Working Group (including experts in medical & surgical oncology, gastroenterology, clinical genetics, genetic counseling, and clinical research), which provides consensus on pedigree analysis, mutation probability, testing strategies, and medical management advice, with interactive discussion from all participants. **Results:** In the first six months, the NVBC presented 17 cases and the Phoenix satellite presented 13 cases. The VC-mediated collaboration resulted in improvement of pedigree and pathology documentation, recommendations for genetic testing and patient management, and identification of potential candidates for chemoprevention and epidemiology research studies. **Conclusion:** VC is a valuable tool for extending GCRA expertise to community-based medical centers and enhances accrual to cancer prevention studies.

**Independent De Novo 22q11.2 Deletions in Two First Cousins with Velocardiofacial Syndrome.** *S.C. Saitta, S.E. Harris, D.A. Driscoll, B.S. Emanuel, D. McDonald-McGinn, E.H. Zackai.* Div Human Genetics, Childrens Hosp Philadelphia, Philadelphia, PA.

Deletions of chromosome 22q11.2 are found in the vast majority of patients with Velocardiofacial/DiGeorge Syndrome (VCFS/DGS). The deletion is estimated to occur in 1:4000 live births, making it the most frequent human microdeletion syndrome. The overwhelming majority of deletions are de novo, with 10% or less inherited from an affected parent. Parental origin studies have previously proven useful to exclude germline mosaicism in an extended family with 2 affected relatives with a 22q11.2 deletion (Driscoll et al, 1997). Here, a second family of 2 unaffected siblings (brother and sister) who each had a child with VCFS/DGS and 22q11.2 deletion as detected by FISH with probe N25. The size of each deletion was determined using FISH with multiple probes from 22q11.2 and both were found to be 3 Mb, the most commonly seen deletion. FISH was normal in the parents of each affected child. We used 6 highly polymorphic microsatellite repeat markers from within 22q11.2 to determine the parental origin of each cousins deletion. This analysis confirmed that in each case, the deletion occurred in a maternally derived chromosome 22. Thus, these were independent de novo deletion events, and we were able to exclude germline mosaicism as the underlying mechanism for affected cousins in this family. These findings underscore the extremely high frequency with which the 22q11.2 deletion occurs in the general population, and demonstrate the important role that PCR-based parental origin determination can have in determining recurrence risks and counseling such families. Although the risk is no higher than the general population, relatives may consider prenatal testing for the 22q11.2 deletion in future pregnancies.

Program Nr: 1126 from the 2001 ASHG Annual Meeting

**Education about etiology and prevention of cleft lip and palate anomalies for Asians families.** *S. Pai, H. Oh, C. Shidara, T. Mosby, M. Tolarova.* Cleft Prevention Program, School of Dentistry, San Francisco, CA.

Cleft lip and palate anomalies are serious and among most common birth defects affecting 1 in every 550 newborns. The prevalence is even higher among American Indians and Asians.

Our goal was a building of public awareness in the field of orofacial cleft (OFC) anomalies in Asian families.

We developed an educational material on etiology and prevention of OFC for Mandarin, Korean and Japanese speaking families. We took into consideration differences in cultural background of each of these ethnically different populations.

**CULTURE DIFFERENCES.** Even though there are obvious cultural differences between individual Asian populations, several common characteristics exist that need to be taken into consideration. Asian men are more active in social-economic activities and women are more responsible for family's diet and health, children's education, and all sorts of matters regarding family. Women are often blamed and also have guilty feelings, if they have a child with a congenital or acquired defect.

**CONCLUSIONS.** It is extremely important to consider cultural differences, customs and taboos in any educational activities focused on prevention of cleft lip and palate anomalies in Asian families. Usual approach, successfully used in Caucasian families, must be modified in order to respect cultural background. Any issues related to family planning must respect leading men's role in majority of Asians families. When prevention protocol requires changes in the life style and dietary pattern, sensitive approach - respecting often deep cultural roots - has to be taken to deliver the message.

The development of the original pamphlet in English and Spanish was supported by MOD Northern California Chapter Community Service Grant.

**Genetic Counseling for Ultrasound Anomalies: Report of a National Genetic Counseling Program.** *D.M. Cutillo, K. Fordham, D. Ramsey, A.E. Donnenfeld.* Dept Clinical Operations, Genzyme Genetics, Philadelphia, PA.

The national prenatal genetic counseling program in Genzyme Genetics encompasses various regions and includes 55 genetic counselors. Starting in January 2001, an integrated clinical computer system began which documents data on all genetic counseling sessions. From January-May 2001, 15,921 patients were seen for genetic counseling. For the indication of abnormal ultrasound, data on 1255 patients was available. The following complete data was gathered on all 1255 patients: maternal age, ethnic background, exposures, family history, and testing and procedure options offered. 92 different types of ultrasound anomalies were identified. The four most common ultrasound anomalies seen were: choroid plexus cyst (24.5%), echogenic intracardiac focus (10.3%), pyelectasis (6.8%), echogenic bowel (6.7%). As part of our clinical practice guideline on ultrasound anomalies, all patients were counseled regarding the option of prenatal diagnostic procedures appropriate for the gestational age, including CVS, amniocentesis, and detailed fetal anatomic survey (if not previously done). All patients' decisions were documented. For invasive prenatal diagnostic procedures (CVS, Amnio) (23.1%) accepted a procedure, (67.6%) declined a procedure, and (9.3%) were undecided about a procedure at the conclusion of the genetic counseling session. Chromosome abnormalities detected: Choroid plexus cyst (4.3% Trisomy 18), echogenic intracardiac focus (2.3% Trisomy 21, 2.3% 47,XX+mar), pyelectasis (2.3% Trisomy 21), echogenic bowel (11.6% Trisomy 21). In most cases of chromosome abnormalities, additional US anomalies were present, there was one case of isolated choroid plexus cyst associated with Trisomy 18, and one case of isolated pyelectasis with Trisomy 21. The introduction of an integrated national clinical computer system, with the capacity to ensure accurate and reliable data input following the genetic counseling session, enhances our ability to: 1) Document patient decisions following the genetic counseling session, 2) Develop specialized education programs for our genetic counselors, 3) Monitor adherence to our clinical practice guidelines, 4) Collect and report data on large numbers of patients.

**Psychosocial impact of genetic testing for tuberous sclerosis complex (TSC).** *B. Clark-Gay<sup>1</sup>, A.J. Tucker<sup>1</sup>, M.A. Assel<sup>1</sup>, D.A. Johnston<sup>2</sup>, K.-S. Au<sup>1</sup>, H. Northrup<sup>1</sup>.* 1) Department of Pediatrics, The University of Texas Medical School; 2) Department of Biomathematics, M.D. Anderson Cancer Center, Houston, TX.

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder with a prevalence of 1/6700. The clinical manifestations are highly variable among individuals, with seizures and mental retardation often causing significant morbidity. Two TSC genes have been identified: TSC1(9q34) and TSC2(16p13). Approximately 60%-80% of individuals diagnosed with TSC have a detectable mutation. Genetic testing for TSC has recently become available on a clinical basis, yet the psychosocial impact of genetic testing in this population remains unexplored.

The study objectives included developing a genetic counseling protocol for disclosing genetic testing results while assessing the impact of the results. Thirty subjects completed a study questionnaire and SCL-90-R before result disclosure and one month following result disclosure. Forty percent of the individuals were adults affected with TSC, and 60% were unaffected parents receiving a result on their affected child. The top three motivations for genetic testing were (1) to understand the cause of TSC, (2) to help family members, and (3) to obtain information about recurrence risk. Reproductive concerns were not listed as a primary reason for testing; however, the data suggest that reproductive decisions might be impacted by the genetic testing results. Unaffected parents receiving results on their affected child did not exhibit a statistically significant change in psychological symptoms after result disclosure. However, affected individuals reported significantly fewer psychological symptoms after result disclosure. The results were statistically significant for a reduction in symptoms of depression ( $p=0.034$ ), hostility ( $p=0.038$ ), and global severity index ( $p=0.026$ ). Our data suggest that genetic testing for TSC does not cause psychological distress, and genetic testing within a genetic counseling setting may result in a reduction of psychological symptoms in affected individuals. The findings may be helpful to genetics providers and consumers to aid in integrating molecular testing for TSC into clinical practice.

**Genetic Counseling for Primary Care Providers: Use of Standardized Patient as a Model for Education in**

**Genetics.** *G. Adamo*<sup>1</sup>, *R.E. Hawkins*<sup>1</sup>, *C.J. Macri*<sup>2</sup>. 1) NCA Medical Simulation Center, Uniformed Services University, Bethesda, MD; 2) Department of Obstetrics and Gynecology, Uniformed Services University, Bethesda, MD 20814.

Advances in the Human Genome Project have accelerated the need for education in medical genetics for all healthcare providers. Experts in the field have recognized that the majority of patient evaluation and care in genetics in the future will be offered by primary care providers. Because the basic education of these healthcare providers may not have included the many new advances in medical genetics, our project addresses the educational needs of this population. We have created a library of standardized patient cases which portray patients with breast, colon and ovarian cancer, polycystic kidney disease, hemochromatosis, cystic fibrosis, and sickle cell anemia among others. The cases offer opportunities for providers to practice and demonstrate their skills in a safe, educational environment. These encounters are used to improve and assess their skills, knowledge and attitudes about medical genetics. For example, in a routine clinic visit the provider is challenged to elicit patient concerns, family history, a three generation pedigree, and the specific history of sickle cell anemia in family members. The National Capital Area Medical Simulation Center was established by the Uniformed Services University in 2000 to enhance the educational process of medical and nursing students, residents and practicing professionals. This state of the art simulation center features a 12-exam room clinical skills laboratory and a computer laboratory area that are used for teaching and assessment activities. The computer laboratory allows web based searches of pertinent medical and genetic information to be used by primary care providers interacting with standardized patients. The providers are instructed to use the web based material and searches, in order to gather information necessary to address specific patient needs. Standardized patients offer a unique opportunity to provide intensive educational experiences for primary care providers in complex multi-disciplinary fields such as medical genetics.

**Parental evaluation of the experience of being informed that a child has a cleft lip and/or palate.** *A.L. Byrnes, M.L. Marazita, N.W. Berk, M.E. Cooper.* University of Pittsburgh, Pittsburgh, PA.

To assess parental satisfaction, differences between parental experiences and preferences with respect to cleft lip and/or palate informing interviews were investigated. A within group comparison of a sample of parents of children with orofacial clefts ascertained from a multidisciplinary craniofacial center was conducted. The Health Professional-Parent Communication Questionnaire (Strauss et al., 1995) was used to obtain information about informing interview experiences and preferences with respect to eleven dimensions of health professional behavior. Descriptive statistics, Wilcoxon matched-pairs signed-ranks analyses, and chi-square analyses were performed. Results revealed that the majority of parents were informed of a child's cleft lip and/or palate at the time of birth (87%) by a physician (87%). These findings were consistent with those reported by Strauss et al. (1995). Parents in this sample wanted informing health professionals to be in greater control of the informing conversation ( $p=0.0033$ ), to show more caring ( $p<0.0001$ ) and confidence ( $p<0.0001$ ), to show more of their own feelings ( $p=0.0140$ ), to give parents more of an opportunity to talk ( $p<0.0001$ ) and show feelings ( $p<0.0001$ ), to make a greater effort to comfort parents ( $p<0.0001$ ), to provide more information ( $p<0.0001$ ), to initiate more of a discussion about the relationship between clefts and mental retardation/learning disabilities ( $p<0.0001$ ), and to provide more referrals to other parents ( $p=0.0353$ ). With the exception of health professionals showing their own feelings, Strauss et al. (1995) also found significant differences between parental experiences and preferences for these dimensions. A positive association between the degree to which parents reported knowing the health professionals at the time of the informing interview, and their satisfaction was identified for ten of the eleven dimensions of health professional behavior ( $0.0001 \leq p \leq 0.0247$ ). Strauss et al. (1995) also found significant associations between knowledge of health professional and six of these behavior dimensions.



**Genetic counseling in hereditary breast/ovarian cancer families in Israel: retention of genetic information and psychosocial impact.** *M. Frydman<sup>1</sup>, M. DiCastro<sup>1,2</sup>, I. Friedman<sup>2</sup>, B. Goldman<sup>1</sup>, E. Friedman<sup>2</sup>.* 1) Genetics Inst, Haim Sheba Medical Ctr, Tel Hashomer, Israel; 2) Oncogenetics Unit, Sheba Medical Center, Tel-Hashomer, Israel.

Genetic counseling for individuals at high risk for developing breast and ovarian cancer (oncogenetic counseling) involves consultees education as the genetic basis of inheritance in cancer, evaluation of cancer risk, psychological evaluation, and genetic testing for germline mutations in BRCA1/BRCA2 genes. In mutation carriers, cancer risk can be objectively verified and even semiquantified, whereas mutation non-carriers may actually be recategorized as "average cancer risk". The long-term psychosocial impact of oncogenetic counseling on consultees and the retention of oncogenetic information are uncertain. We retrospectively interviewed 155 women who underwent oncogenetic counseling in a single medical center in Israel in 1996 and 1998, 29 BRCA1/BRCA2 mutation carriers and 126 non-carriers. A validated questionnaire that evaluated self-reported distress and anxiety symptoms before and after counseling, as well as the retention of relevant genetic information (e.g., individual and offspring cancer risk, early detection schemes) one and three years after the initial consultation was filled by study participants. Overall, oncogenetic counseling had a minimal effect on anxiety related symptoms. Mutation carriers report of anxiety-associated symptoms such as sleeplessness, "bad mood", more frequently than non-carriers following oncogenetic counseling. As expected, 61.8% of carriers and only 30% of non-carriers accurately remembered the genetic risk and preventive and early detection schemes. We conclude that no overt adverse psychosocial effects can be attributed to oncogenetic counseling, and that there is an ongoing need to emphasize oncogenetic information to high-risk individuals.

**Epidemiological impact of genetic counselling and predictive testing for myotonic dystrophy.** *J. Mathieu, C. Tremblay, C. Prevost.* Dept Neurology, Complexe Hosp de la Sagamie, Chicoutimi, PQ., Quebec, Canada.

The prevalence of myotonic dystrophy type 1 (DM) is very high in the Saguenay-Lac-Saint-Jean (SLSJ) region, a geographically isolated region in the northeastern part of the province of Quebec (Canada). In SLSJ, a program of genetic counselling for DM patients and their families was introduced in 1981 and a program of predictive testing is offered to the population since 1988. In order to determine the effect of genetic counselling and predictive testing on the prevalence of the DM phenotypes, we compared the demographic characteristics and the phenotypes of the DM patients over the last 15-year interval (1985-2000). During this period, the median age of the DM patients increased from 34.5 years to 44.0 years ( $p < 0.00001$ ). The median age of the congenital, childhood and classic DM patients increased respectively from 9 to 24 years ( $p < 0.001$ ), 24 to 33 years ( $p = 0.01$ ) and 35 to 44 years ( $p < 0.001$ ). In this time interval, the proportion of patients with a congenital or a childhood phenotype remained stable but the proportion of patients with a classic phenotype decreased from 75% to 61% and, the proportion of patients with a mild phenotype increased from 6% to 20% ( $p < 0.001$ ). In the last 5 years, among the 63 newly diagnosed patients, 43% had a mild DM phenotype detected only by DNA analysis. The reasons for the significant ageing of the DM population in SLSJ region are complex and include a low natality rate throughout the Quebec population, a reduction in births at risk due to genetic counselling and an increase in the number of mildly affected patients often recognized at an older age only by predictive testing. The shift in the distribution of the DM phenotypes toward mildly affected individuals is expected to accelerate in the future.

**Patient Confidentiality vs. Duty to Warn At-Risk Relatives: The Experiences of Genetics Counselors. R.B.**

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When a patient refuses to inform relatives of their risk for genetic disease, a genetic counselor (GC) is faced with conflicting ethical obligations. On one hand, there is the obligation to respect and protect their patient's right to privacy. On the other hand, there is the obligation to prevent harm and promote the welfare of family members. This suggests a responsibility to warn at-risk relatives, even without the patient's consent. Unfortunately, there is little to guide the GC when faced with this situation. There is no legal standard, and the guidelines of various societies leave considerable room for subjective interpretation. Furthermore, there is little literature on our clinical experience with this, as most studies have been based on theoretical situations. Interestingly, most have found that GCs were less likely than medical geneticists to overrule patient confidentiality. To examine the actual clinical impact of this issue, we surveyed ~1,300 National Society of Genetic Counselor (NSGC) members to examine their experience with this conflict. GCs were invited to answer a survey that was posted on the Internet or available at the 19th NSGC Conference. The survey addressed GCs' experience with patients' refusal to share genetic information with at-risk relatives, and the factors that lead to their decision whether to warn or not. 119/259 (46%) respondents had a patient refuse to notify an at-risk relative. Only 24 (9%) reported seriously considering warning without consent, and only 1 did. This case had special circumstances, as the at-risk relative was also a patient of the GC. Three factors consistently made the GC less likely to warn: patient's potential emotional reaction, relative and patient's emotional relationship, and the chance that the relative was aware of the disease by another means. These results suggest that in clinical experience this conflict is usually resolved by respecting patient autonomy. While several factors may have contributed to this, emotional factors were the most critical in deciding not to warn. Respondents also indicated they viewed notifying relatives without consent outside the GCs role and responsibility.

**Counseling concerns with cystic fibrosis mutation analysis.** *G.A. Jervis<sup>1</sup>, R. Montenegro<sup>2</sup>, B.G. Kousseff<sup>1</sup>*. 1) Regional Genetics Program, Univ South Florida, Tampa, FL; 2) Bayfront Maternal Fetal Medicine St. Petersburg, FL.

Cystic fibrosis (MIM#219700) is an autosomal recessive disease caused by mutations in the cystic fibrosis conductance regulator gene (CFTR) located on chromosome 7q31. Manifestations of the disease include pancreatic insufficiency, meconium ileus, chronic bronchopulmonary infections and elevated sweat chloride concentrations. To date, over 900 CFTR mutations have been identified. We present an African American family referred during their third pregnancy because they have two sons with a clinical diagnosis of cystic fibrosis. The oldest son (MC) had meconium ileus and surgery for duodenal atresia. He uses a gastric tube and is on enzyme therapy. The younger son (JC) is asymptomatic but allegedly had a positive sweat test. Parents are healthy and have no family history of cystic fibrosis. Molecular studies performed at Genzyme Genetics laboratory revealed: Father- DF508/(-); Mother- DF508/D1270N; MC- DF508/DF508; JC- DF508/D1270N; Amniocytes- DF508/D1270N. Thus, the mother, JC and fetus have the same genotype. The pregnancy resulted in a healthy male with a normal sweat test. If the offspring had all three mutations, then the maternal mutations would have been in cis. There are reports that double mutant CFTR alleles are more common than expected (Human Molecular Genetics, 1995,4:1169). The three DF508/D1270N individuals are most likely compound heterozygotes. The phenotype of DF508/D1270N has been reported with normal pulmonary and pancreatic function (World J Urol, 1193:11,82), which explains the asymptomatic state of these individuals. However, there are reports that males with DF508/D1270N have congenital bilateral absence of vas deferens (CBAVD). So, the two sons with DF508/D1270N genotype will be evaluated for CBAVD and counseled accordingly. This family illustrates the complexity of genotype-phenotype correlations in cystic fibrosis and demonstrates that certain combinations of CFTR mutations can result in asymptomatic phenotype but may have consequences for fertility.

**Risk Reduction Behavior in Individuals and Families at High Risk for Breast/Ovarian Cancer Syndrome: The UCSF Cancer Risk Program Experience 1996-2000.** *J.L. McLennan, E.B Crawford, A.E. Toland, J.L. Ziegler, D. Tripathy, L.J. Esserman.* Cancer Risk Program, UCSF Comprehensive Cancer Center, San Francisco, CA.

**Introduction.** The Cancer Risk Program at UCSF provides genetic counseling and testing for individuals at high risk of developing breast/ovarian cancer syndrome within the framework of a prospective study aimed at reducing their cancer risk. Since its inception in 1996, 1038 potential clients have contacted the Program of whom 463 agreed to genetic counseling. We offered genetic testing for BRCA1 and BRCA2 mutations to counseled individuals whose risk of being a carrier exceeded 10%; (BRCAPRO software). **Method.** In a cross-sectional design, we analyzed the outcomes after counseling and testing 170 individuals who enrolled in the Program between 10/96 and 12/00. Group I tested positive (n=80) for a deleterious mutation in BRCA1 or BRCA2. Group II (n=90) was tested and found NOT to have known deleterious mutation in either BRCA1 or BRCA2 (Ashkenazi founder analysis, n = 47, full sequence analysis, n= 43). No true negatives and no subjects with variants or polymorphisms were included in either group. **Results.** In an interim outcomes analysis (n= 70), both groups reported high level adherence (>95%) to recommended screening guidelines for both breast and ovarian cancer. Over 80% reported positive lifestyle changes (improved diet, more exercise, and less stressful cancer worries). 47% of Group I chose prophylactic contralateral or bilateral mastectomy and the majority (95%) were later satisfied with the decision. 72% of Group I chose prophylactic ovarian surgery [BRCA1 91% v BRCA2 72%] and after careful histopathology review, two mutation carriers had Stage I cancer in their ovaries. 95% of both groups shared information about genetic test results with other family members, but only half of families with a mutation carrier proband have opted for further genetic testing. **Conclusion.** Genetic counseling and testing in our experience is associated with positive risk reduction behavior in the majority. Probands should be encouraged to inform other relatives at risk to amplify the cancer prevention effect of genetic testing in families.

**Evidence that Depression-Screening is Useful in Prenatal Genetic Counseling.** *M. Jurek<sup>1</sup>, J. Edwards<sup>1</sup>, C. Lovell<sup>1</sup>, C. Singletary<sup>1</sup>, V. Vincent<sup>1</sup>, C.M. Wolpert<sup>2</sup>.* 1) Genetic Counseling Program, University of South Carolina, S.C. 29203; 2) Duke University Medical Center Durham, N.C. 27710.

Depression commonly occurs in women during pregnancy. If left untreated, depressive symptoms have been associated with an increased risk for preeclampsia, low birth weight, and post-partum depression. Surprisingly, in the United States there are no formal depression-screening programs targeted towards prenatal patients. Genetic counselors are uniquely poised to offer depression screening given that the typical prenatal genetic counseling session includes psychosocial assessment. The objective of this study is to examine whether genetic counselors recognized symptoms of depression in the prenatal advanced maternal age population. We hypothesized that a standardized depression-screening questionnaire would be most effective in identifying depression in this patient population. Prenatal patients were asked to complete a standardized depression inventory, the Beck Depression Inventory (BDI-II) in the waiting room prior to their prenatal genetic counseling session. A board-certified, prenatal genetic counselor that was blinded to the results of the BDI-II completed a psychosocial assessment for depression after the session. A supervising genetic counselor scored the patient's BDI-II and compared the two methods for concordance. Women identified as depressed were informed of their results and referred to their health care provider by the supervising genetic counselor. Of the thirty-five patients who volunteered for the study, 10 (28.57%) screened positive for depression on the BDI-II. Only three of these 10 patients were identified by their prenatal genetic counselor as having symptoms of depression, representing a 30 % concordance in between the BDI-II score and the genetic counseling assessment. This pilot data suggests that a significant percentage of depressive symptomatology in AMA prenatal patients may not be apparent to the genetic counselor using routine methods. Incorporating a formal depression-screening tool such as the BDI-II may be useful in a prenatal setting.

**Assessment of the Effectiveness of Genetic Counselling by Telephone Compared to a Clinic Visit.** *K.K. Sangha<sup>1</sup>, A. Dircks<sup>2</sup>, S. Langlois<sup>2</sup>*. 1) Dept. of Medical Genetics, University of Alberta Hospital, Edmonton, Alberta, Canada; 2) Dept. of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

Maternal serum screening, also known as triple screen, is used during pregnancy to assess the risk of carrying a fetus with specific chromosome abnormalities or an open neural tube defect. A blood test administered between 15 and 20 weeks of pregnancy measures the levels of three proteins in the maternal serum to calculate the risk to the fetus of having trisomy 18, trisomy 21, or open spina bifida. All pregnant women in British Columbia (BC), Canada are eligible to have maternal serum screening and the test is optional. All women who screen positive (approximately 8% of all women tested) are eligible for genetic counselling and are offered amniocentesis. The purpose of this study is to determine what differences (if any) exist in patients understanding and/or anxiety when genetic counselling for a positive triple screen result is conducted in person versus over the telephone. Currently, all genetic counselling sessions for positive triple screen result are conducted in person in the Provincial Medical Genetics Programme at BC Children's and Women's Health Centre in Vancouver. In this study each patient who agreed to participate was given the choice to have her genetic counselling session in person or over the telephone. Through the use of a brief written post-counselling questionnaire, each patient was assessed for her understanding of the information presented in the session, and her anxiety regarding her risk. In this small pilot study, there were no statistically significant differences detected in patients' understanding or anxiety when genetic counselling for a positive triple screen for Down syndrome was conducted by telephone versus in person.

**The Impact of DNA Analysis on Genetic Counselling for 276 Retinoblastoma (RB) Families.** *J. Sutherland*<sup>1,2,4</sup>, *J. Anderson*<sup>2</sup>, *S. Richter*<sup>2</sup>, *B. Gallie*<sup>1,2,3,4</sup>. 1) The Retinoblastoma Program, Ophthalmology, Hospital for Sick Children; 2) Solutions by Sequence, Inc; 3) Ontario Cancer Institute/Princess Margaret Hospital, University Health Network; 4) University of Toronto, all in Toronto, Ontario; Canada.

Clinical care and genetic counselling for RB families is enhanced by knowledge of the precise mutation in the proband. Without DNA analysis, counselling consists of presenting several scenarios, each dependant on when the predisposing RB1 mutation occurred. Statistics were generated from a FileMaker Pro database used by *Solutions*. Mutations were identified and reported for 276 probands (specificity >85%). Within our own institution, counselling was provided for 182 probands' families. Mutation detection was particularly useful in counselling parents of isolated cases of unilaterally affected children by discriminating somatic, non-germline RB from heritable disease. After two RB1 mutations identified in tumor but not in blood, 89%(86/97) probands then counselled to have reduced tumor risk for the other eye and for other lifetime cancers. Despite the lack of family history, 11%(11/97) of unilateral probands had germline mutations. Counselling and screening then proceeded as for bilateral families. Of 125 non-familial, bilaterally affected probands, 96%(69/72) of tested parents were normal, suggesting *de novo* mutations. Probands' sibs are tested for the mutation because of the possibility of germline mosaicism in the parent. Statistics predict 50% of offspring of adult, bilateral survivors are predisposed to RB tumors. DNA analysis identified the children at risk for tumor development. The children who are not, could safely opt out of examinations under anesthetic and clinical examinations could be reduced or eliminated. Thus for 91% of families, DNA analysis reduced relatives' risk to 1 in 20,000, sparing sibs and cousins from invasive clinical screening under anesthetic. In conclusion, the ability to alleviate uncertainty in counselling strongly supports DNA testing for RB. DNA analysis identifies which scenario to address and personalizes risks for relatives allowing greater decision-making power for the families.



**Clinical interpretation and recommendations for patients with a variant of uncertain significance in BRCA1 or BRCA2: a survey of genetic counseling practice.** *N.M. Petrucelli<sup>1</sup>, N. Lazebnik<sup>1</sup>, K.M. Huelsman<sup>2</sup>*. 1) Department of Medical Genetics, Henry Ford Hospital, Detroit, MI; 2) Division of Human Genetics, Children's Hospital Medical Center, Cincinnati, OH.

**Purpose:** To document current practices in breast cancer genetic counseling and to identify areas of variability in practice for patients with a variant of uncertain significance (VUS) in the BRCA1 or BRCA2 gene. **Methods:** Three hundred and seventeen active members of the National Society of Genetic Counselors (NSGC) were sent an invitation via electronic mail to participate in an online questionnaire. The online questionnaire consisted of 22 questions divided into 3 sections: clinical experience with VUS, clinical meaning of VUS, and risk perceptions and clinical recommendations for given clinical situations involving a VUS. Some open-ended questions were provided to allow respondents greater flexibility in their answers and the opportunity to give qualitative comments. **Results:** Of the total 295 members meeting eligibility criteria, 57 (19.3%) responded. During the pre-test counseling session for a BRCA risk assessment patient, the vast majority of counselors (80.7%) mention VUS as a possible test result. However, only 49.1% report having given such a result to their patients. Furthermore, only 63.2% (36) felt as though their patients understood the meaning of a VUS test result. When asked to conclude the implication of a VUS and make medical management recommendations, the responses were varied. Nevertheless, a good proportion of counselors expressed the importance of testing other family members to help clarify the probands risk and aid in medical management issues. **Conclusion:** While the recent recommendations by the American College of Medical Genetics suggest standards for the interpretation of sequence variations, they do not provide guidelines for making clinical recommendations based on these variations. The results of this study reveal significant diversity in the personal interpretation of a VUS test result, ultimately leading to various clinical recommendations. This suggests a need for clinical management standards as well.

**Pretest Counselling Post Testing: Experiences of Providing Genetic Counselling Services to Participants in a Population Based Study of the Prevalence and Penetrance of BRCA1 and BRCA2 Mutations in Ovarian Cancer.**

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Genetic counselling for hereditary breast and ovarian cancer has typically involved in-depth pretest counselling sessions for those individuals eligible to have genetic testing for mutations in the BRCA1 and BRCA2 genes. It is only following such thorough counselling where issues such as insurability, employability, and psychological impact are discussed, that a blood sample for genetic testing can be drawn. Once genetic testing results are available, the patient returns to the clinic for another visit to discuss the full implications of the results with respect to their clinical management.

Here we describe the experience and results of offering genetic counselling to a series of women with ovarian cancer that provided blood samples for genetic testing for mutations in the BRCA1 and BRCA2 genes. These women were offered genetic testing as part of a research protocol to determine the prevalence and penetrance of germline BRCA1 and BRCA2 mutations in women with ovarian cancer.<sup>1</sup> Genetic counselling services were provided only to those women electing to receive their test results.

Of the 1024 eligible women that were diagnosed with epithelial ovarian tumors in Ontario between January 1995 through December 1996, 649 (63%) agreed to provide blood samples. To date, 124 (19%) of these women have accepted a referral for genetic counselling in order to receive their test results, 58 of whom were seen in our center. We evaluate here the effectiveness of this form of genetic counselling in comparison to more traditional methods. We also evaluate the uptake of genetic testing for hereditary breast and ovarian cancer as compared to that found in the routine cancer genetics clinic.

1. Risch et al 2001. *AJHG* 68:700-710.

**Participant satisfaction with genetic counseling/testing and psychosocial adjustment at a university-based family cancer registry.** *J.L. Seldon, L.C. Kwan, J.E. Bower, P.A. Ganz.* Jonsson Comp Cancer Ctr, Univ California, Los Angeles, Los Angeles, CA.

In 1998, the UCLA Familial Cancer Registry and Genetic Evaluation Program was established to identify individuals and families at increased risk for familial cancer. One Registry objective is to serve as a resource of subjects whose informed consent participation may contribute to genetic, behavioral, and other hypothesis driven research protocols. All Registry participants are asked to contribute biological specimens such as blood/tissue and complete the following baseline and annual follow-up data: demographics, hazardous work exposures, psychological measures, quality of life, diet, physical activity, tobacco/alcohol use, medical history, cancer screening, other health practices, and detailed pedigree. A second objective is to provide genetic counseling/testing to individuals at high risk for cancer predisposition mutations. Psychosocial and genetic knowledge data are collected prior to genetic counseling, at time of blood collection, and at 1, 6, and 12 months post-testing. Satisfaction with genetic counseling/testing is collected 1 month post-testing. Overall, Registry participants (n=169) did not show elevated levels of psychological distress. Scores on standardized measures of depressive symptomatology, anxiety, and quality of life all fell well within normal limits. Level of distress about genetic counseling and testing (n=47) was also low overall. However, we did see an increase in test-related distress among individuals who tested positive; distress levels increased at 1 month post-testing, and continued to rise at 6 months in this group. In contrast, test-related distress decreased among individuals who tested negative. The increase in test-related distress was followed by an increase in depression scores among participants testing positive, suggesting that issues related to cancer risk may have had a broader impact on mood as participants contemplated post-testing decisions such as prophylactic surgery and chemoprevention. Regardless of test results, the participants were highly satisfied with the genetic counseling/testing experience.

**Levels of genetics knowledge and distress: A comparison of cancer survivors and unaffected women in a BRCA1/2 testing program.** *K.A. Schneider, A.B. Chittenden, L.M. DiGianni, A.F. Patenaude, R. Gelman, J.E. Garber.* Dana Farber Cancer Inst, Boston, MA.

The BRCA1/2 testing experience might be different for cancer survivors compared to women who have never had cancer. We compared genetics knowledge and depression and anxiety scores for 86 breast or ovarian cancer survivors and 72 unaffected women in a BRCA1/2 testing program. Demographics were similar for both groups including education and income levels. All participants had at least a 10% chance of having BRCA1/2 mutations. Positive results were reported to 38 women (19 cancer survivors, 19 unaffecteds) and negative results were reported to 104 women (55 cancer survivors, 49 unaffecteds). Variant results were excluded. Participants completed a 20-item knowledge questionnaire at baseline and 2 weeks post-results. Depression and anxiety subscales of the Brief Symptom Inventory (BSI) were completed at baseline and 4 months post-results. **RESULTS:** Knowledge scores were higher among unaffected women than cancer survivors both at baseline (66.52 vs 57.24,  $p=.02$ ) and post-results disclosure (72.05 vs 63.76,  $p=.005$ ). Knowledge scores rose for both groups from baseline to post-results. Overall depression and anxiety scores for cancer survivors and unaffected women were not different at either measurement, but cancer survivors showed improved scores from baseline to 4 months post-results [anxiety mean score: 48.7 to 45.6, depression mean score: 50.1 to 47.7]. Sorting the groups by test results, we found that cancer survivors with negative results had improved anxiety and depression scores and cancer survivors with positive results had improved anxiety scores. Unaffected women had unchanged depression and anxiety levels regardless of result. **SUMMARY:** Cancer survivors were less knowledgeable than unaffected women about testing both at baseline and 2 weeks after results disclosure, although knowledge scores improved for both groups. Cancer survivors distress scores decreased with results whereas test results did not alter scores in unaffected women.

**Genetic Screening Pocket Facts Card: An educational tool for primary care practitioners.** *S.S. Brooks, J.D. Krogh, N.J. Zellers, E. Nikezic-Ardolic.* Human Genetics, New York State Inst Basic Res, Staten Island, NY.

For many medical disciplines pocket cards are available containing critical information practitioners can review while providing patient care. Based on this model we developed a *Genetic Screening Pocket Fact Card* containing practical genetic information for clinicians. Topics covered included conditions in the eastern Jewish population, fragile X mental retardation syndrome, hemoglobinopathies and cystic fibrosis. The cards measured 7 by 14 inches when open and 3.5 x 7 inches when folded. They were laminated for durability. In addition to the card, a patient information sheet was developed with information about genes, recessive inheritance, screening, prenatal testing, carrier frequencies and additional information sources. These tools were distributed to 6500 primary care physicians in New York City. The physicians were asked to return a 6 question survey. Besides indicating their type of practice, they assessed on a scale of 1-5 (least-most) how frequently genetics was discussed with their patients; whether the information presented was clear; whether the pocket guide would be useful to them; how much they would be willing to pay for additional copies; and whether the patient information was useful for their practice. Preliminary results of the survey show that internists are least likely, pediatricians and family practitioners somewhat likely and obstetricians most likely to discuss genetics with their patients. All groups felt that the information presented was clear. Obstetricians indicated that they would use the guide most often, followed by family practitioners, pediatricians, and internists. Most groups found the patient information sheets useful as well. Despite the positive response to the information 36% of those surveyed would not be willing to pay for additional copies. Those willing to pay would spend \$1. However, we did receive several requests for multiple additional copies. Overall, there was a very positive response from clinicians for this novel genetic educational tool. *This project was funded by a community service grant from the Greater N.Y. March of Dimes.*

**Genetics education in New England family practice residencies.** *W.G. Feero<sup>1</sup>, P. Haddow<sup>2</sup>, D.K. Olson<sup>1</sup>.* 1) Maine-Dartmouth Family Practice Residency Program, Augusta, ME; 2) Foundation for Blood Research, Scarborough, ME.

Family practice physicians are likely to play a major role in delivery of genetic health care to rural and underserved communities in the United States. In 1999 the American Academy of Family Practice(AAFP) established training guidelines for genetics in family practice residencies. One year after the establishment of these guidelines we surveyed the program directors of 17 New England family practice residencies to determine the extent to which genetics is a part of resident training. Furthermore, we sought to determine factors that might affect the presence of genetics training in the curricula. 13/17 of programs responded, three reported having a formal curriculum in genetics. Those with a formal curriculum reported 5-10 hours(1/3) and >10 hours(2/3) of genetics training during the three years of residency training. Those without formal curricula all estimated less than 10 hours of genetics training. Within the limitations of the survey size, there was no correlation between the size of the program, accessibility of medical geneticists to the program, priority ascribed to genetics in training by program directors, or the level of health care provided at the primary teaching hospital and the presence of a formal curriculum. Most commonly, genetics training was reported to occur in the context of pediatric rotations. 10/13 programs reported using a genetics screening questionnaire for new obstetric patients, 1/13 programs reported using a genetics screening questionnaire for new adult patients. Three conclusions may be drawn from this survey. First, based on this sample, a minority of family practice programs have formally integrated genetics into residency training. Second, the presence of genetics resources does not influence the presence of a genetics curriculum, suggesting that other factors (competing training priorities, for example) may determine the presence of a curriculum. Finally, these results suggest family medicine training emphasizes pediatric and obstetrical aspects of genetics, and may not be preparing residents to deal with adult-onset genetic disorders.

**Incorporating Cultural Assessment Approach to Regimens Employed in Conducting Genetic Family Studies.**

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Current approaches in conducting genetic family studies (GFS) focus on the biological aspects of genetic diseases, while the sociocultural dimensions are given secondary importance. Understanding the sociocultural aspects of the enrollment process provides important insight into how subjects interpret and value their participation in GFS. We have developed and applied a new instrument using cultural assessment approach (CAA) to our recruitment procedures in the Family Investigation of Nephropathy and Diabetes (FIND) study at UTHSCSA. The CAA was developed based on data collected during semi-structured interviews with 32 subjects. 20 diabetic nephropathy (DN) probands with a family history of diabetes and 12 relatives. Subjects mean age was 53 years. 44% were males and 56% were females. 64% were Mexican-Americans, and 52% had a high school education. Applying the CAA takes about 30 minutes. The instrument is composed of 33 closed-ended questions regarding subjects' (1) ethnomedical beliefs about the genetic nature of DN (2) attitudes towards participation in the FIND study and (3) awareness of ethical issues and risks related to participation. CAA allows building rapport between recruiters and subjects, identifying subjects' attitudes towards participation and exploring sociocultural factors and ethical issues related to participation. Integrating the CAA to our recruitment procedures in FIND has increased our efficiency by targeting efforts towards the willing subjects while addressing the concerns of the undecided. We administered the CAA to 50 DN probands to evaluate their attitudes towards participating and inviting their relatives to the FIND study. 29 probands were willing, 6 were undecided and 15 were unwilling. We contacted 35 probands and enrolled 31 families in the FIND study (Family enrollment rate is 88.5%). By identifying and excluding the unwilling subjects, CAA has saved at least 30% of the recruiters' efforts. We will discuss the theoretical paradigm behind the CAA and present some cases to illustrate how the CAA enhances (1) awareness of genetic diseases and (2) enrollment in GFS yet meet participants needs. This work was funded by NIDDK.

**Increasing physicians' knowledge of hereditary hemochromatosis using a novel web-based delivery method.** *C.I.*

*Barash*<sup>1</sup>, *M.R.G. Taylor*<sup>2</sup>, *C. Pacifici*<sup>3</sup>. 1) Genetics, Ethics & Policy, Boston, MA; 2) University of Colorado Health Sciences Center, Denver, CO; 3) Northwest Media Inc.

**Background:** Genetics knowledge is advancing rapidly, but integration of this information into clinical medical practice has not increased at the same pace. Sizeable resources have been committed to educating primary care providers about the growing relevance of genetics to clinical medicine, yet few if any training programs have demonstrated significant improvements in knowledge and understanding. **Methods:** We constructed and evaluated a prototype Web site designed to promote diagnostic acumen by increasing physicians' knowledge about Hereditary Hemochromatosis (HH), <http://hh.northwestmedia.com>. The training used a self-paced multimedia approach to present state-of-the-art knowledge on HH. The approach was suitable for training practitioners with various knowledge bases, skill levels, and learning styles. We will demonstrate the site and present results from our evaluation of the approach. **Results:** Fifty-seven physicians completed a 43-item knowledge questionnaire administered on the site, pre- and post-intervention. The sample included training physicians in internal medicine and family practice; 38% of the sample was female, and 17% were from non-White ethnic groups. The majority of participants reported that they had not spent any time learning about the "new genetics." Roughly one-third reported managing an HH patient previously. Using a 2-tailed paired difference t-test, results indicated that subjects made highly significant improvements in knowledge from pre- (Mean= 73.8%, SD=6.82) to post-intervention (Mean=80%, SD=5.05),  $t(56)=6.59$ ,  $p=.001$ . Subjects had the most difficulty with items in two specific areas: the genetics of HH, particularly genotype/phenotype associations, and treatment/management. Users reported a broad range of preferred learning styles. Overall satisfaction with the site was excellent. **Conclusions:** Medical institutions in the U.S. and abroad struggle with similar cost and time constraints to achieve efficient and effective medical education. This study documents the efficacy of an innovative tool in the field of medical learning practices.



Program Nr: 1147 from the 2001 ASHG Annual Meeting

**Generation of Case Studies for the Teaching of Medical Genetics.** *K.A. Fleiszar, R.C. Martin, P. Johnson.* Biol, Col Sci & Mathematics, Kennesaw State Univ, Kennesaw, GA.

From 1983 until 1993 over 500 genetic consultations were completed and recorded by KAF. These consultations provided an excellent source of case studies for the teaching of medical genetics. With support from KSU's Student Assistants in Learning and Teaching (SALT) program, two students (RCM and PJ) were hired to transfer this information into a database using the software program *Progeny 2000*. Prior to beginning the data entry, issues of confidentiality were addressed. Selected cases were then entered into pedigree form using this program. Once entered, student case studies were generated by modification of these original pedigrees. Thirty of these case studies were used spring 2001 in the upper level Medical Genetics course (Biology 3327). The student was required to obtain information concerning his/her assigned case study from the instructor. Students submitted a paper addressing the recommendations to be made to the consultand of this family relative to the specific RFR (reason for referral). At a minimum, these recommendations included appropriate pre- and postnatal diagnostic tests, explanations of those tests and their risks, risk assessment for family members immediately impacted by the RFR and explanations of the expected prognosis for the disorder involved. In addition to the summary paper, one other disorder manifested in the family was chosen for a detailed poster presentation. At the end of the semester, use of these case studies was assessed. Were they an effective way of teaching about heritable and non-heritable birth defects? What difficulties were encountered by the students in interpreting family information? What problems were encountered in conveying information about risks and recommendations? Results of this assessment are reported. Additional cases are being recorded and will be used for Medical Genetics as well as General Genetics (Biology 3300), Biology of Cancer (Biology 4630) and Chromosome Preparation and Analysis (Biology 4427). Biology 3327 and 4427 are required courses for the baccalaureate program in Cytogenetic Technology at KSU.

Program Nr: 1148 from the 2001 ASHG Annual Meeting

**Design and Outcomes of an Intensive Course in Cancer Risk Assessment.** *K.R. Blazer, D.J. MacDonald, J. Choi, R.A. Nedelcu, S. Sand, J.N. Weitzel.* Dept of Clinical Cancer Genetics, City of Hope Comprehensive Cancer Center, Duarte, CA.

Cancer risk assessment (CRA) is a new discipline that requires knowledge of both oncology and genetics. There is a growing need for clinicians cross-trained in these specialties. The City of Hope (COH) *Center for Cancer Genetics Technology Transfer Research* designed and conducted an intensive two-week cancer genetics training course for masters-educated genetic counselors and advanced practice nurses, funded by the California DHS Cancer Research Program. A central goal was to increase access to competent CRA services in outlying California communities, while expanding the sphere of COH *Cancer Screening & Prevention Program* research. Twelve participants (one-half from each discipline) were competitively selected on the basis of academic merit, demonstrated need in their community and institutional support. The CME/CEU-accredited course (70 hours) provided didactic and case-based learning modules, workshops, surrogate patient counseling and wetlab sessions in a format designed to enhance interdisciplinary interactions. Students completing the course have access to the program's interdisciplinary staff and other clinicians through the *Cancer Genetics Link* web-board, a novel internet discussion board for continued practice-based learning. Questionnaires were administered prior to and immediately after the course, and again six months after course completion. Knowledge of clinical cancer genetics increased by 34 percent at the immediate post-course timepoint. Additional impact measures included assessment of effectiveness of teaching methods, participant satisfaction and impact of knowledge gain on practice patterns through the six-month post-course timepoint. Overall, the course was well received and resulted in additional clinicians with screening-level competence in cancer risk counseling, practicing in areas with defined need for cancer risk assessment services.

**Multidisciplinary Genetic and Prenatal Diagnosis Education Via Internet in a Rural State.** *S. Barringer<sup>1</sup>, K. Icke<sup>1</sup>, B. Butler<sup>1</sup>, T. DuBose<sup>1</sup>, B. Karczeski<sup>2</sup>.* 1) University of Arkansas for Medical Sciences, Departments of Ob/Gyn and Radiology, Little Rock, AR; 2) Johns Hopkins University, DNA Diagnostic Laboratory, Baltimore, MD.

Facing a shortage of sonographers in our state, especially high-risk obstetrics and prenatal sonographers, the genetic counselors and radiology instructors at UAMS developed a 2 credit, internet based course entitled Advanced Obstetric Sonography, Genetics, and Pathology. Designed for senior Diagnostic Medical Sonography (DMS) students at UAMS, the course included 16 Web-based lectures, weekly bulletin board class discussions, reading assignments, problem-based case questions, and 2 examinations. Rural obstetric and genetic patients in states such as Arkansas do not always receive the highest quality ultrasounds during their pregnancies, due to the limited educational background of some ultrasonographers/physicians. This course was piloted in our DMS program as a means of reaching and improving rural healthcare education. Additionally, this program enabled many students completing off-site clinical practicums to remain at those sites while still taking courses. We also wanted to examine the feasibility of using this method of education to enable health-care providers obtain continuing education credits. Lectures were posted using WebCT software. Topics included basic genetics, diagnosis of fetal ultrasound anomalies, embryology of these birth defects, teratogen risks, role of the sonographer on the obstetrical care team, and psychosocial counseling in cases of fetal abnormalities. Various relevant links were included in these lectures for further reading. The "bulletin board" allowed for open discussion on these topics. After their final examination, students were asked to evaluate several components of the course. The overall content/method of the course was rated a 4.2 out of 5 on the Likert scale. Several important benefits and limitations regarding this method of teaching were noted. In the future, we hope to develop more distance genetic education courses for the underserved Great Plains region, as well as offer continuing education via distance education methods.

Program Nr: 1150 from the 2001 ASHG Annual Meeting

**Evaluating a Multimedia Program 'Medical Genetix'.** *S.A. Metcalfe*. Education Unit, MCRI, and Dept Paediatrics, The University of Melbourne, Royal Children's Hospital, Parkville, Australia.

An interactive multimedia program, Medical Genetix, has been developed, which features 7 more common single-gene and chromosomal disorders that often form the clinical framework for understanding genetic principles within medical and allied health courses. After evaluation during developmental stages, the program has been further evaluated as a self-directed learning resource (SDL) and for use within computer-based practical classes. Overall, feedback has been extremely favourable with many positive comments about the usefulness and interactive aspects of the program. At the University of Melbourne, students undertaking a Graduate Diploma of Genetic Counselling have used sections of the program as an SDL tool. The mean score for 'helpfulness in assisting learning' was 8.7 out of 9, with 5 conditions studied by the current cohort. Students especially liked the self-testing questions and the clinical relevance, whilst their knowledge improved post-use when tested. All medical students (undergraduate) used parts of the program in 1st and 2nd year within timetabled prac classes. In one prac, students gave a mean rating of 7.7 out of 9 for its usefulness in helping their understanding of karyotyping and molecular pathogenesis of chromosomal conditions. Interestingly, only about 28% of medical students had used the program earlier as an SDL resource, others often citing lack of time as their reason. With a different cohort, when the student guide to the problem-of-the-week (muscular dystrophy) stated that the program contained material assessable in exams, use of the program increased to about 60%. Of these students, 73% gave the program a rating of between 7-9 out of 9 as a helpful source of information, compared with 62% who gave textbooks the same rating. In general, at the University of Melbourne, medical students may not often use multimedia programs unless they are timetabled and/or compulsory, a trend seen not only for Medical Genetix. Nevertheless, students who did use Medical Genetix have found it very useful as a learning resource and it seems to be particularly helpful for graduate students, such as those studying genetic counselling.

**Theory and Practice in Teaching Human Genetics to Undergraduate, Non-Science Majors.** A.M. Hott<sup>1</sup>, C.A. Huether<sup>1</sup>, J. McInerney<sup>2</sup>, H. Bender<sup>3</sup>, C. Christianson<sup>1</sup>, J. Jenkins<sup>4</sup>, A. Wysocki<sup>5</sup>, G. Markle<sup>1</sup>, R. Karp<sup>1</sup>, R. Fowler<sup>6</sup>. 1) University of Cincinnati, Cincinnati, OH; 2) Natl. Col. for Health Prof. Ed. in Gen., Baltimore, MD; 3) University of Notre Dame, Notre Dame, IN; 4) Swarthmore College, Swarthmore, PA; 5) Natl. Inst. of Nursing Res., Bethesda, MD; 6) San José State University, San José, CA.

Based upon a Human Genetics Education (HGE) workshop at the 1999 Am. Soc. of Human Genetics (ASHG) meeting, the I & E committee appointed an *ad hoc* committee to review HGE at the undergraduate collegiate level. Specifically, the charge was to produce a series of recommendations about content and pedagogy for undergraduate courses that should include significant content in human genetics. The committee's initial focus has been on Introductory Biology courses for Non-science majors. The committee developed a list of 6 main concepts and 43 sub-concepts it felt should be taught in such courses. An Internet survey instrument was then designed for course instructors to determine the degree to which these concepts and sub-concepts were being taught. The total hours spent on each concept were determined, as were the relative importance and emphasis an instructor attached to each sub-concept (1-5 scale with 5 highest). A total of 357 responses from both 2-year and 4-year institutions were obtained. An average of 15.6% (14.4 hrs) of the time spent in all introductory, non-science major biology courses studied is devoted to the area of genetics/human genetics. Of the six main concepts, the highest average time spent was on Transmission of the Genetic Material (3.0 hrs), with 1.8 hours committed to Genetics and Society (fifth lowest). In all sub-concepts except one, a statistically significant higher *importance* was reported compared to the *emphasis* individuals were able to give that sub-concept in the course. This disparity between importance (3.9) and emphasis (3.4) represents a significant challenge for improving HGE in non-science major biology courses, since one of the logical causes for this difference is time. Instructors recognize the importance of genetics as a major component of biology education but are unable to give the emphasis it demands.

**Expanding the multi-disciplinary team in South Africa - Advanced Genetic Nurse.** *E. Pietersen<sup>1</sup>, C. Cupido<sup>1</sup>, D. Kibel<sup>2</sup>, R.S. Ramesar<sup>1</sup>.* 1) Human Genetics, University of Cape Town, Cape Town, Western Cape, South Africa; 2) Psychiatry, University of Cape Town, Cape Town, Western Cape, South Africa.

The clinical implication of information emerging from the Human Genome Project has compelled nurses to review, as well as expand, their scope of practice. Nursing staff involved in genetic research programmes have the advantage of "hands on" experience with the discipline of genetics. These nurses end up developing expertise on an ad hoc basis and are obliged to become leaders in the practice of genetic nursing. The task of the genetic nurse is going to become more complex with forays into the genetics of complex inherited disorders. Since 1997 the Department of Human Genetics at the University of Cape Town has been involved in research related to bipolar affective disorder. To date we have recruited 810 individuals from 139 families. This recruitment prefaced an intensive molecular investigation into the biological basis of bipolar disorder. Involvement in this psychiatric genetic research programme highlighted (a) the need for nurses to interrogate the family history of psychiatric subjects / patients from a genetic perspective (over and above the known clinical psychiatric perspective) (b) the fact that relatives of individuals with psychiatric disorders, in this particular study bipolar disorder, are at higher risk for other psychiatric disorders (ie. major depressive disorder, alcohol abuse, schizophrenia) and (c) the fact that bipolar disorder exists very likely as part of a spectrum of disorders which may reflect common biological pathways. Within the scope of practice of psychiatric nursing, the need for an emergence of professional psychiatric nurses with an understanding of clinical and genetic heterogeneity, molecular genetics, and inheritance of complex disorders, cannot be ignored. This background will create a new niche for an advanced psychiatric genetic professional nurse who will be able to be a patient advocate with regard to issues around genetic counselling, pharmacogenetics, as well as medical ethical issues pertaining to, for example genetic services (predictive testing in particular) and research.

**A Statewide Project To Educate and Raise Awareness About "The New Genetics": The CGEP Project in Vermont.** *R.R. Wallace-Brodeur<sup>1</sup>, E. Mahoney<sup>2</sup>, K. Richardson-Nassif<sup>1</sup>, L. Burke<sup>1</sup>, D. Yandell<sup>1</sup>.* 1) University of Vermont, Burlington, VT; 2) Saint Michael's College, Colchester, VT.

A major hope for the Human Genome Project is that it will provide important new insights into the role of inheritance in human health, behavior and disease. This knowledge will lead to opportunities for improved disease management, prevention and public health genetics, but will also create the need for rapid and effective public and professional education related to new genetic technologies. The Community Genetics and Ethics Project (CGEP) is an NHGRI-ELSI funded educational project designed to educate and raise awareness about 'the new genetics' across Vermont. The statewide project has partnered with community organizations and existing state networks to facilitate access to the broadest possible audience including professional, lay, and special-interest groups. CGEP is organized in a "concentric circles" model, with initial contact occurring in intensive 2 to 3 day retreats. Retreat participants are then called upon to facilitate interaction with broader audiences via local, community-based discussion groups and public "town meeting" style forums.

To date, CGEP has coordinated 12 multi-day retreats for diverse professional groups, 25 book discussion groups, and town meeting style evening forums around the state. All events target the specific audience using a mixture of lecture, panel, and small group discussion formats. A survey instrument is used to assess the project's impact and significant changes in knowledge and attitude questions have been seen. Target audiences have a high level of interest but varying levels of concern related to ELSI issues, and misconceptions are abundant in the rural population we serve. Access and "mode of entry" into specific subgroups are a major challenge, but our experience suggests that targeting preexisting well-organized networks or community organizations will be most successful. Barriers to raising awareness and educating the general population about ELSI issues and the "new genetics" can be overcome by sustained and carefully coordinated educational interventions.

**Evaluating Men's Interest in Interactive Technology Use to Facilitate Prostate Cancer Education and Risk Counseling.** *D. Watkins Bruner, A. Parlanti, S. Raysor, S. Mazzone, T. Sargent.* Population Science, Fox Chase Cancer Center, Philadelphia, PA.

Men at increased risk for prostate cancer, as defined by family history and race, have unique information needs regarding prostate cancer risk factors and screening. Participants attending the Prostate Cancer Risk Assessment Program (PRAP) at Fox Chase Cancer Center currently receive a standardized educational process with a health educator to help them achieve an understanding of their prostate cancer risk and to prepare them for health care decisions related to that risk. While this information is critical for making informed decisions at the time of screening, the format is often inconvenient for participants, labor intensive for staff, and does not suit all learning styles. Purpose: To conduct a pilot study to determine if men in a prostate cancer risk assessment program would be interested in using technology to receive educational information about prostate cancer risk and screening. Method: Participants in the PRAP were asked to complete a survey about their current access to and use of certain technology including personal computers. They were also asked about their preferences in modalities for receiving information about health, prostate cancer risk and prostate cancer screening. Results: Of the 21 respondents, the mean age was 50 with 47.6% Caucasian, 47.6% African American and 4.8% other. 85.7% reported a family history of prostate cancer. 81% reported owning or having access to a personal computer with 66.7% using the personal computer one or more hours per week. 71.4% indicated an interest in receiving prostate cancer information using an interactive computer program and 76.2% responded that they would feel comfortable answering questions about their personal risk for prostate cancer using various forms of technology. Conclusion: Men at increased risk for prostate cancer that are seeking prostate cancer screening express an interest in interactive media and technology to learn about prostate cancer risk and screening. This provides incentive to develop and implement an interactive multimedia program to aid men in understanding their risk for prostate cancer.



**Unintended messages: The ethics of teaching genetic “dilemmas”.** *H.C. Gooding<sup>1</sup>, B. Wilfond<sup>1</sup>, K. Boehm<sup>2</sup>, B.B. Biesecker<sup>1</sup>*. 1) Medical Genetics Branch, NHGRI, Bethesda, MD; 2) Office of Communication and Education, NIDCR, Bethesda, MD.

The teaching of bioethics often uses challenging cases. However, these cases may cause harmful messages to be received by certain communities. One such example is the "Case of Dwarfism," presented shortly after the discovery of the common mutation for achondroplasia in 1994 and subsequently disseminated in bioethics and educational materials. This case describes a couple, both affected with achondroplasia and expecting a child, who plan to terminate the pregnancy if prenatal diagnosis reveals the fetus is of average stature. It is often employed as a teaching case for health professionals, designed to stimulate debate over their role as the gatekeepers of access to genetic testing. It is more broadly applied to compel audiences to examine their views on reproductive freedoms and the limits of parental autonomy. While this case may challenge stereotypes about the appropriate uses of prenatal diagnosis, it potentially fosters a more subtle stereotype about the community it intends to serve. It presents people with achondroplasia as motivated to make reproductive decisions only by their perceptions of their physical features. However, a recent study of people affected with achondroplasia revealed that the majority considered knowing the prenatal genetic diagnosis of average stature unimportant and that only 2% would consider termination based on this finding. Using this case to challenge the view of what makes for a "healthy baby" may actually deny that those with achondroplasia simply share the common parental desire for healthy children of any stature. Furthermore, it may cause health care professionals to make assumptions about the desires of clients that would inhibit open discussion about the individual needs of people considering genetic testing. While such cases remain important teaching tools in genetics, we should be aware of our own inadvertent messages and their potential for creating discriminatory attitudes.

**Knowledge of genetic discrimination in healthcare practitioners in a large metropolitan area.** *R. Nedelcu<sup>1</sup>, P. Mantha<sup>1</sup>, J. Yu<sup>1</sup>, S. Sand<sup>1</sup>, J. Choi<sup>1</sup>, B. Schwerin<sup>2</sup>, K. Blazer<sup>1</sup>, S. McCaffrey<sup>1</sup>, D. McDonald<sup>1</sup>, J. Weitzel<sup>1</sup>.* 1) Clinical Cancer Genetics, City of Hope Cancer Ctr, Duarte, CA; 2) Cancer Legal Resource Center, Western Law Center for Disability Rights and Loyola Law School, Los Angeles, CA.

While a great deal of discussion has taken place in the medical community regarding genetic discrimination, little effort has been made to formally assess the knowledge and beliefs of those who influence access to genetic counseling and genetic testing, such as primary care physicians and nurses. A 35 item questionnaire on cancer risk knowledge and attitudes was administered to 134 participants at an all-day symposium on cancer risk, screening and prevention. To address knowledge of state and federal laws and beliefs about genetic discrimination and the extent of their effect on referral practices, we included a 10-item survey, with clinical vignettes which was completed by 129 participants. Most of the respondents were physicians (34%), nurses (37%) and genetic counselors (14%). Overall, 51% believed carriers of a genetic mutation have difficulty obtaining insurance, and 24% would not encourage genetic testing despite the presence of a family history of cancer. An interesting finding was that 56% were concerned about genetic discrimination in a woman with breast cancer at 32, but 66% would not be concerned about this issue for the woman's unaffected sister. This is an important observation, since if the woman with cancer experiences discrimination, it is more likely due to her diagnosis, while the greatest potential for insurance discrimination may be among unaffected carriers of cancer-predisposing mutations. Almost 90% of respondents believed the potential for genetic discrimination exists regardless of protective state or federal legislation. Additionally, 25% believed documented cases of genetic discrimination of unaffected carriers existed despite a lack of published cases. We conclude that a great deal of misinformation exists regarding insurance discrimination based on genetic testing for cancer predisposition, and an educational effort directed at healthcare professionals is imperative to address this.

Program Nr: 1157 from the 2001 ASHG Annual Meeting

**The reproductive behaviour in families with Fabry disease after genetic counselling. A 20 year follow-up.** S.A. Sorensen, L. Hasholt, K. Rosenberg. Dept. of Medical Genetics, University of Copenhagen, Copenhagen N, Copenhagen, Denmark.

In Denmark six families are known with Fabry disease. These families were traced and examined in the 1970's. All families were given genetic counselling and were informed about the possibility of prenatal investigation.

In the 20 year period after the genetic counselling 34 pregnancies occurred in heterozygotes. Prenatal investigation was done in 21 cases which revealed ten male fetuses with a Fabry mutation. In all but three of these cases the pregnancy was terminated. In the remaining cases five were male fetuses with a normal alpha-gal A gene, and six were female fetuses which all were born.

Among the children born of heterozygotes who did not have a prenatal investigation three boys were born with a Fabry mutation and in further three boys the gene status is currently unknown.

Our results show that genetic counselling and prenatal investigation in families screened for Fabry disease has a considerable effect on the prevention of new cases, but still not all cases are prevented.

**Genetic counseling for BRCA testing: Considerations for young female clients.** *A.A. Parlanti, A. Naumer, E. Ross, M.B. Daly.* Population Science, Fox Chase Cancer Center, Philadelphia, PA.

The potential risks and benefits of BRCA counseling and genetic testing can vary among client populations. As a result genetic counselors must adapt the content of sessions to benefit the client. Young women, 30 years and younger, are one example of a small but distinct population of clients. Currently there are limited reports regarding the lifestyle, screening behaviors, cancer risk perceptions, and anxiety levels for this group of women. The present study aims to determine the number of female participants ages 30 and younger in Fox Chase Cancer Center's Family Risk Assessment Program and to identify similarities and differences compared to older participants. Of the 1712 individuals who have participated from 1991-2001, 262 (15.3%) were women ages 30 or younger at the time of their initial phone call (average of 26.3yr). All completed an initial phone survey and health history questionnaire and participated in individual risk counseling sessions. 82.6% had a familial or hereditary pattern of breast or ovarian cancer in their maternal family and 29.5% in their paternal family. Compared to the women older than age 30, there were significant differences in level of education, marital status, religion, employment status and income level. As expected, older women reported significantly higher rates of mammography and breast biopsy, although rates of BSE were similar. While both groups thought their risk of getting breast or ovarian cancer exceeded that of other women their age; the younger women rated their risk significantly higher than the older women. However the younger women had significantly less frequent thoughts about developing breast cancer and those thoughts had less effect on their mood and daily activities. Additional information, including genetic knowledge, lifestyle, and future preventative planning was also analyzed. In general, we propose that this group of women differ physically, emotionally and socially from older women seeking genetic counseling for BRCA and that by understanding these differences genetic counselors can modify counseling by addressing the unique needs of these young women.

**Genetic & Psychological Implications for Adolescent Females at Risk for Familial Breast Cancer.** *M. Cappelli*<sup>1,3</sup>, *Y. Korneluk*<sup>3</sup>, *S. Verma*<sup>4</sup>, *A. Hunter*<sup>2</sup>, *E. Tomiak*<sup>4</sup>, *J. Allanson*<sup>2</sup>, *C. DeGrasse*<sup>5</sup>, *L. Corsini*<sup>5</sup>, *L. Humphreys*<sup>3</sup>. 1) Psychology, Children's Hospital of Eastern Ontario, Ottawa, ON; 2) Genetics, Children's Hospital of Eastern Ontario, Ottawa, ON; 3) Psychology, University of Ottawa, Ottawa, ON; 4) Ottawa Regional Cancer Centre, Ottawa, ON; 5) Ottawa Women's Breast Health Centre, Ottawa, ON.

Adolescent daughters at risk for familial breast cancer represent a group at increased risk for emotional problems. To date, however, this population has been understudied and this study included the largest sample group of adolescent daughters of breast cancer patients to date. We compared 62 mother-daughter pairs in which the mother had been treated for breast cancer (BC group) to 51 families from the general population (GP). (Mean age of women=45 years, and mean age of daughters=15 years; averaged 3 years post-treatment for Stage I or II BC). Participants completed an assessment battery of standardized and validated measures to assess psychological functioning, breast cancer, and genetic issues. Results indicated that adolescent daughters of breast cancer patients had higher levels of knowledge about breast cancer risks ( $p<.05$ ). Whereas 28% of adolescents in the BC group were more likely to have heard about breast cancer gene testing, only 11% of GP adolescents group had heard of it ( $p<.05$ ). BC group adolescents perceived the treatments for breast cancer as less effective and significantly more debilitating than girls in the general population ( $p<.05$ ). They also reported higher levels of concern about breast cancer than did adolescent girls in the general population ( $p<.05$ ). Overall, the BC group did not differ from the GP group on measures of psychological adjustment and family functioning. Additional analyses of the sub-group of breast cancer patients and their daughters indicated that maternal HQL and family functioning were important predictors of adolescent reports of their psychological adjustment. These results suggest that positive family functioning can help to buffer the negative effects maternal illness on adolescent social functioning. Genetic attitudes were independent of how well mothers and daughters coped.

**Sequencing the human genome: An assessment of media coverage and public reactions.** *B.A. Bernhardt, E. Tambor, N.A. Holtzman, G. Geller.* Genetics/Public Policy Studies, Johns Hopkins Medical Inst, Baltimore, MD.

Media coverage of the June 2000 announcement of the sequencing of the human genome was enormous. Not much is known, however, about public reactions to the sequencing. Because the mass media is a primary source of health information for most people, we analyzed the content of media reports of the genome sequencing. We also assessed public awareness of and reactions to the announcement. Within two weeks of the announcement, we conducted a random digit dialing phone survey of Maryland residents. Responses to open-ended questions were coded for qualitative and quantitative analysis. 58% of the 407 survey respondents had heard about the announcement, nearly all through the media, with television cited as the major information source. Caucasians, men, respondents over 40 and those with some college education were more likely to have heard. The majority reported a positive initial reaction, with expectations for improved treatment or prevention of disease. Most thought such advances would occur within the next ten years. 61% of respondents mentioned at least one negative outcome of genome sequencing, most commonly privacy/discrimination, and 14% thought that sequencing might lead to human cloning. African Americans were more likely than Caucasians to report a negative reaction ( $p < .001$ ), primarily related to religious concerns and mistrust of scientists. We also conducted a content analysis of 49 media reports cited by survey respondents as their source of information about the genome sequencing. Among the reports analyzed, newspaper/wire reports focused primarily on what had been done and who was involved while television reports focused more on medical and ethical implications. Ethical concerns most frequently covered included discrimination, gene patenting and "designer babies". These findings confirm that much of the public is aware of genetic advances and hold mixed reactions to them, with optimism about future medical benefits tempered by concern about possible risks and abuses. There is a need for continued public discourse, including through the media, to address concerns and clarify misperceptions regarding the Human Genome Project.

**Providing information at the point of care: Educational diagnostic reports from a genetic testing service**

**provider.** *L.M. Goos<sup>1</sup>, I. Silverman<sup>1</sup>, L. Steele<sup>2</sup>, T.L. Stockley<sup>2</sup>, P.N. Ray<sup>2</sup>.* 1) Dept. of Psychology, York University, Toronto, Ontario, Canada; 2) Paediatric Lab Medicine, Hospital for Sick Children, Toronto, Ontario, Canada.

Genetic test requisition by non-geneticists is on the rise, and is expected to increase as the availability and public awareness of genetic testing grows. The need for physician education in genetics has been identified in numerous studies, and practice-based educational initiatives have generally been found to be most effective. The Molecular Genetics Laboratory at the Hospital for Sick Children has developed a program to provide physician-directed information at the point of care. Physicians requesting molecular testing receive the Diagnostic Report as well as an Information Sheet describing the requested test. Semi-structured telephone interviews were conducted to gather information about the clarity of the Diagnostic Report and Information Sheets, the utility of the Information Sheets in physicians' practice, and the use of the report during consultations with patients. Fifty-five interviews were conducted, representing 45% of physicians contacted during the study period. Those interviewed had between 6 and 43 years in practice, and ordered less than 1 test per month to more than 40. 45% of the interviewees were pediatricians or pediatric specialists; 29% were genetic specialists. The remaining 26% were specialists in other areas or were general or family practitioners. 43% of those interviewed were unfamiliar with some of the information on the Information Sheet. The test methodology and sensitivity were most often identified as novel information. 52% of pediatricians or pediatric specialists were unfamiliar with some aspect of the Information Sheet, despite being the highest consumers of testing services in this sample. Pediatricians also rated the Information Sheet highest in terms of its usefulness in their practice, followed by genetic specialists and other physician categories. Overall, physicians confirmed the utility of the Information Sheet as an educational tool in practice, and in other non-patient educational activities in which they are involved.

**Possessive versus non-possessive eponyms: What is the current status?** *B.N. Chodirker, A.E. Chudley.* Departments of Pediatrics and Child Health & Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB.Canada.

For years, geneticists have advocated against the use of the possessive form of an eponym as the author neither had nor owned the disorder (Lancet. 1975 1;1(7905):513). Therefore, Down syndrome is preferred to Down's syndrome. Although OMIM uses the non-possessive form exclusively, Dr. McKusick considered the use of the possessive form optional for some disorders such as Huntington disease due to the long usage of the possessive form or the difficulty in pronouncing some non-possessive eponyms. The purpose of this study was to determine the relative frequency of the possessive versus non-possessive forms of eponyms. Medline searches were conducted for articles published in English in the years 1996-2000 inclusive. Article titles were searched for the following: "Down syndrome"(DS), "Turner syndrome"(TS), "Marfan syndrome"(MS), "Huntington Disease"(HD), "Parkinson Disease" (PD) and "Alzheimer Disease" (AD) as representative examples of syndromes and diseases with eponyms. Searches were also done for the possessive form of these eponyms. The percent usage of the non-possessive form was as follows: DS = 58%, TS = 60%, MS = 67%, AD = 15%, PD = 4%, HD = 17%. The use of the non-possessive form was more common with syndromes: 60% vs. 12% ( $p < 0.001$ ). The pattern of use depended on the country of publication. For example, articles published in the United States were more likely to use the non-possessive form than articles published in England i.e. 74% vs. 42% ( $p < 0.001$ ) for syndromes and 18% vs. 3% for diseases ( $p < 0.001$ ). Genetics journals i.e. journals with "Genet" in their title, were more likely than non-genetics journals to use the non-possessive form i.e. 93% vs. 56% ( $p < 0.001$ ) for syndromes and 37% vs. 11% for diseases ( $p < 0.001$ ). These data show that the use of the non-possessive form of the eponym is not yet widely accepted and is not yet universally used by geneticists. We believe that the continued use of both systems creates confusion. We would recommend that journal editors adopt a uniform system for the use of eponyms. Geneticists should be advocating for the use of the non-possessive form of eponyms.



**Knowledge about hereditary breast cancer among early-onset breast cancer survivors.** *S. Miesfeldt*<sup>1</sup>, *W.F. Cohn*<sup>2</sup>, *M.E. Ropka*<sup>2</sup>, *S.M. Jones*<sup>3</sup>. 1) Dept Internal Medicine; 2) Dept Health Evaluation Sciences; 3) Cancer Center; Univ Virginia, Charlottesville, VA.

Little is known about hereditary breast cancer (HBC) knowledge among women with breast cancer (BC). This study assessed if women with early-onset BC (<50 years): 1) have the necessary HBC knowledge to address the need for genetic counseling; and 2) show differences in knowledge based on HBC risk. Participants responded to two mailed questionnaires. The Knowledge, Attitudes and Beliefs Questionnaire (KABQ) evaluated understanding of BC risk factors and HBC knowledge. The Family History Questionnaire (FHQ) assessed whether participants had personal or family histories that met HBC risk criteria. Women diagnosed < age 50 years during 1994-97 were recruited from 34 Virginia hospitals. Based on FHQ responses, women were categorized as having suspected HBC vs. presumed sporadic breast cancer (SBC). Of 314 respondents, 273 (87%) returned both questionnaires. Average age of respondents was 47.5 years. 91% were Caucasian, 7% African-American and 2% Asian-American. 138 met HBC risk criteria; 135 were presumed to have SBC. Most knew common BC risk factors, including family history of BC. 31% recognized family history of non-breast cancers as a BC risk factor. 62% knew that prophylactic mastectomy does not completely eliminate BC risk. Most recognized that not all women carrying a BC mutation will develop BC (72%) and that men can develop BC (96%). In contrast, the majority selected "I don't know" for several items concerning characteristics of HBC, including: potential risk transmission through a father (58%); risk of early-onset BC among mutation carriers (55%); increased risk of male BC in mutation carriers (71%); increased risk for other types of cancers (61%); and bilateral BC (62%). Knowledge regarding HBC did not vary between women at risk for HBC and those with presumed SBC. These data show limitations in HBC knowledge among early-onset BC survivors, and highlight the need for educational materials regarding HBC for these women.

**GeneTests and GeneClinics: genetic testing information for a growing audience.** *R.A. Pagon<sup>1,2</sup>, P. Tarczy-Hornoch<sup>1</sup>, M.L. Covington<sup>2</sup>, P.K. Baskin<sup>1</sup>, J.E. Edwards<sup>1</sup>, M. Espeseth<sup>1</sup>, C. Beahler<sup>1</sup>, T.D. Bird<sup>1</sup>, B. Popovich<sup>3</sup>, C. Nesbitt<sup>1</sup>, C. Dolan<sup>1</sup>, K. Marymee<sup>1</sup>, N.B. Hanson<sup>1</sup>, W. Neufeld-Kaiser<sup>2</sup>, G. McCullough Grohs<sup>2</sup>, T. Kicklighter<sup>1</sup>, C. Abair<sup>1</sup>, A. Malmin<sup>1</sup>, M. Barclay<sup>1</sup>, R.D. Palepu<sup>1</sup>.* 1) University of Washington, Seattle, WA; 2) Children's Hospital and Regional Medical Center, Seattle, WA; 3) Xenon Genetics, Vancouver BC.

Over the last decade, genetic testing has moved steadily out of research venues and into medical practice. The audience for genetic testing information has become international and expansive, and includes healthcare providers, patients, educators, policy makers and media. This presentation describes the growth and usage of two companion NIH- and HRSA-funded genetic testing information resources, GeneTests ([www.genetests.org](http://www.genetests.org)) and GeneClinics ([www.geneclinics.org](http://www.geneclinics.org)), which reflect this trend. Since 1993, GeneTests (né Helix) has grown from a national genetic testing laboratory directory of ~110 listings to an international directory of clinical and research labs (~500 labs, ~800 diseases), a US clinic directory (~950 clinics), an educational/teaching resource, and a source of summary data reflecting trends in genetic testing of increasing interest to educators, policy makers and the media. GeneClinics, founded in 1997 as an expert-authored, peer-reviewed, disease-specific information resource relating genetic testing to patient care, now contains more than 110 full text entries. The combined resources are accessed ~1,000,000 times/year by a diverse audience, of which about 20% is non-US and about 35% is the public. For non-geneticist healthcare providers, diverse search strategies help identify testing available in their specialties, and a hierarchical display of search results aids in differential diagnosis.

**State of clinical genetics and genetics education in academic internal medicine departments.** *M.R.G. Taylor.*  
University of Colorado Health Sciences Center, Denver, CO.

The genetics revolution has led to the recognition that many adult-onset disorders have a genetic basis. Clinical genetics, historically a pediatric-based discipline, has growing importance in the medical care of adult patients. The present number of board certified (BC) geneticists with training in adult medicine is insufficient to meet the growing needs of this population. Accordingly, internal medicine-trained physicians (internists), who deliver much of the medical care to adult patients, may be called upon to evaluate and treat patients with genetic illnesses. Previous authors have shown that internists know and understand less about genetic principles and conditions than their peers (e.g. pediatricians, obstetricians, family practitioners). Academic internal medicine departments (AIMDs) train the bulk of practicing internists in the U.S. The responses of AIMDs to the challenges raised by advances in adult clinical genetics have not been well characterized. A survey tool, querying chairpersons of AIMDs about their responses to advances in genetics, was sent to 155 AIMD chairpersons. 58% (90/155) were returned with usable data. Only 18% (16/90 responses) of the AIMDs had faculty members who were BC geneticists; 11% (11/90) had only one BC geneticist; 24% (21/87) employed genetic counselors. 72% (64/89) offered formal didactic teaching about specific genetic conditions for training internists. Of this group, 44% (28/64) included instruction taught by BC geneticists. Thus, only 31% (28/89) of AIMDs surveyed offer formal didactic genetics programs taught by BC geneticists. 93% (82/88) of chairpersons agreed that the majority of genetic diagnoses in adult patients are made by internists who are not well trained in genetics. 77% (68/88) agreed that all AIMDs should include faculty that have formal training in clinical genetics and 36% (31/87) indicated that they were trying to hire a BC geneticist in the next two years. **These data highlight that BC geneticists appear to have a limited role currently in AIMDs in terms of providing clinical service and educational expertise. There is interest in increasing the involvement of BC geneticists in AIMDs..**

**Knowledge and attitudes of Israeli Arab adolescents regarding consanguineous marriages - implications for educational programs.** *T. Shohat, O. Romano-Zelekha, L. Jaber, M.S Green.* Israel Center for Disease Control, Ministry of Health, Tel Hashomer, Israel.

Consanguineous marriages are very common in the Israeli-Arab population. It was shown that the prevalence of cousin-cousin matings is as high as 40%. As a result, the main cause of infant mortality in this population is congenital malformations. Several educational programs were established in attempt to reduce this phenomena. In a national survey of 2933 Arab schoolchildren age 16-17 (comprising 20% of all Arab children at this age group) we evaluated the knowledge and attitudes regarding consanguineous marriages. 45.8% of the study participants scored less than 60 on the knowledge questionnaire. Male sex, lower parental education, and being engaged at the time of the study were independently significantly associated with poor knowledge. Although 80% knew that consanguineous marriages could affect the health of the offspring, only 50% understood what congenital malformations were. 73.8% of the study participants expressed attitudes against consanguineous marriages. Male sex, lower parental education, having a relative with congenital malformation, being engaged at the time of the study, and poor knowledge of the possible consequences of consanguineous marriages were significantly associated with attitudes supporting such marriages. 30% of those engaged at the time of the study were engaged to first cousins. 50% of those who thought that getting married to a relative was an important custom that should be followed, reported that they would have done so even knowing the increased risk for health problems to their offspring. The results of this study demonstrate the need to increase the level of knowledge of adolescents regarding consanguineous marriages. However, since consanguineous marriages have deep roots in the Arab population, efforts must be greatly directed to educate individuals of the possibilities of preconception and prenatal testing for various diseases.

**Effective International Collaboration in Rare Disease Research.** *P.F. Terry<sup>1</sup>, S.F. Terry<sup>1, 8</sup>, A.S. Marais<sup>2</sup>, I. Pasquali-Ronchetti<sup>3</sup>, C. Boyd<sup>4</sup>, E. Johnson<sup>5</sup>, T. Le Roux<sup>6</sup>, L. Bercovitch<sup>7</sup>.* 1) PXE International, Inc, Sharon, MA; 2) University of Cape Town, South Africa; 3) University of Modena, Italy; 4) University of Hawaii, Honolulu, HI; 5) Barrow Neurologic Institute, Phoenix, AZ; 6) Lasalle College, Newton, MA; 7) Brown University, Providence, RI; 8) Genetic Alliance, Washington, DC.

Lay advocacy groups accelerate and focus research on a rare disorder. Lack of data about its manifestations, natural history and epidemiology limits research on a disorder. Often the limited number of individuals with the disorder limits the funding possibilities and makes the acquisition of patient samples and data difficult. Further, the agenda of researchers and participants can diverge in ways that ultimately delay progress toward a treatment and cure. Pseudoxanthoma elasticum (PXE) is a rare genetic disorder. PXE International, a lay advocacy organization founded in 1995, initiates, supports and funds research. Also providing support for affected members and their families, it has focused and accelerated research. Establishment of various offices and programs in 21 countries throughout the world has been critical to providing genetic services and supporting research. Empowering the individuals in various countries and cultures requires an investment of resources in the laboratories of these communities. The PXE International Blood and Tissue Bank has branches in Italy and South Africa. The results of efforts made by PXE International are a 15 laboratory consortium, a multidisciplinary approach forging collaborations amongst many specialists, a focused agenda which accelerates research in the service of the interests of the patient population, and increased congressional awareness for real outcomes of medical research funding. The organization is also uniquely able to safeguard confidentiality by acting as a 'firewall' between researchers and participants. Genetic lay advocacy using this model can greatly benefit both research and patient populations, furthering both the common and divergent goals of each.

**Clinical and Genetics Difficulties in Huntingtons disease Reduced Penetrance Alleles.** *A. Sanchez<sup>1</sup>, C. Badenas<sup>1</sup>, T. Marcos<sup>2</sup>, E. Muñoz<sup>3</sup>, D. Jimenez<sup>1</sup>, M. Mila<sup>1</sup>.* 1) Genetica, Hosp Clinic, Barcelona, Catalonia, Spain; 2) Psicologia, Hosp Clinic, Barcelona, Catalonia, Spain; 3) Neurologia, Hosp Clinic, Barcelona, Catalonia, Spain.

Huntingtons disease (HD) is a progressive neurodegenerative disorder characterized by involuntary movements, cognitive disturbances and emotional problems and inherited as an autosomal dominant trait with an average age of onset within the fourth decade of life. It was generally accepted that the disease was 100% penetrant, but nowadays it is not so clear. The molecular defect consists in a CAG repeat expansion in the IT15 gene. In normal chromosomes the CAG repeats range from 9 to 29 whereas they are expanded beyond 41 repeats in HD chromosomes. The range between 36-41 CAGs is considered of reduced penetrance and the risk of developing the disease is uncertain. We have detected 80 patients with alleles of reduced penetrance in the screening of 250 HD Spanish families: 68 of them (32%) are clinically affected and 12 were studied in presymptomatic testing. The mean age of onset in the reduced penetrance group was 52.5, which is higher than the age of onset in our HD population (43.3). We will like to point out that 22% of patients with alleles in this range were considered apparently sporadic. These results indicate the difficulty in the clinical diagnosis of HD when a reduced penetrance allele is present, due to its atypical clinical manifestations, specially in HD patients with late onset and in sporadic cases. We will like to highlight the difficulty of genetic counseling in this range.

**Complex Segregation Analysis of Exercise Heart Rate and Blood Pressure at Baseline and in Response to 20 Weeks of Endurance Training: The HERITAGE Family Study.** *P. An<sup>1</sup>, I.B. Borecki<sup>1</sup>, T. Rankinen<sup>2</sup>, L. Perusse<sup>3</sup>, A.S. Leon<sup>4</sup>, J.S. Skinner<sup>5</sup>, J.H. Wilmore<sup>6</sup>, C. Bouchard<sup>2</sup>, D.C. Rao<sup>1</sup>.* 1) Washington University School of Medicine, St. Louis, MO; 2) Pennington Biomedical Research Center, Baton Rouge, LA; 3) Laval University, Quebec, Canada; 4) University of Minnesota, Minneapolis, MN; 5) Indiana University, Bloomington, IN; 6) Texas A&M University, College Station, TX.

Major gene effects for HR and BP at 50 W and 80% of VO<sub>2</sub>max at baseline and their chronic changes in response to 20 weeks of endurance training were assessed in 99 White families in the HERITAGE Family Study. Exercise HR and BP at baseline were adjusted for the effects of age and BMI within fathers, mothers, sons, and daughters, respectively. The training responses were adjusted for the effects of age, BMI, and their respective baseline values. We found baseline HR at 50 W was influenced by a major recessive gene effect and a multifactorial effect, which accounted for 30% and 27% of the variance, respectively. Its training response was found to be influenced by a major dominant gene effect, which accounted for 27% of the variance. These major gene effects were independent of the effects of smoking, VO<sub>2</sub>max, and the resting HR levels. No significant genotype specific interaction effects with age, sex, and BMI were found. No major gene effects were detected for exercise BP. Instead, we found baseline BP at 50 W and 80% VO<sub>2</sub>max, and the training responses at 50 W were solely influenced by multifactorial effects, which accounted for about 50%, 40%, and 20% of the variance, respectively. No familiarity was found for training responses in HR and BP at 80% VO<sub>2</sub>max. In addition, the major gene effects for exercise HR were assessed in a pooled data with a small sample of Black participants in HERITAGE. Similar major effects were found, but the transmission from parents to offspring became ambiguous, which would suggest sample heterogeneity. In conclusion, submaximal exercise HR levels at baseline and in response to endurance training were influenced by putative major loci with no genotype specific covariate effects, in contrast to multifactorial components for exercise BP.

**Degree of common genetic determination of peak bone mineral density at the hip and spine in women.** *D.L. Koller, M.J. Econs, S.L. Hui, M. Peacock, C.C. Johnston, Jr., T. Foroud.* Indiana Univ Sch Medicine, Indianapolis, IN.

Fractures of the femoral neck and the lumbar spine account for a substantial portion of the \$13 billion per year spent in the U.S. toward treating the consequences of osteoporosis. Studies of twin and family data have established the heritability of peak bone mineral density (BMD), a strong predictor of fracture risk, at both the femoral neck and lumbar spine; however, it is unknown to what degree common genetic factors affect BMD at these two sites. We have addressed this question in independent sibling pairs from our collection of 351 sets of Caucasian sisters. BMD was measured using DEXA (Lunar DPXL) at the lumbar (L2-L4) spine and femoral neck for each subject. Regression model-fitting was used to adjust the observed BMD values for age and body weight. Genetic model-fitting was performed with the Mx software package (Neale et al, 1994). Univariate models included a genetic component and an environmental component. The bivariate model included common genetic and environmental components acting on both traits, and also unique genetic and environmental components for each trait. Heritability in this sample, computed using the univariate models in Mx, was 83.4% for lumbar spine and 66.8% for femoral neck. These are in strong agreement with values reported previously by our group and others. The standard Pearson correlation between these two BMD measures, which ignores the family relationship of the subjects, was 56.5% for this data set, also in agreement with previously reported values. The bivariate models as fitted with Mx showed that a common set of genetic factors explained 36.7% of the variability of lumbar spine BMD and 34.0% of the variability in femoral neck BMD. Thus, these common factors contribute nearly equal portions of the total variability of each measure; however, common genetic factors contribute a greater portion of the genetic variability of femoral neck BMD ( $34.0\%/66.8\%=50.9\%$ ) as compared with lumbar spine BMD ( $36.7\%/83.4\%=44.0\%$ ). These results strongly support the hypothesis that common genetic factors influence BMD at multiple skeletal sites, but that there are also important unique genetic factors acting at each site.



**SR-B1 variants associated with HDL cholesterol levels in three populations.** *J.J. McCarthy<sup>1</sup>, S. Lewitzky<sup>1</sup>, A. Permutt<sup>2</sup>, B. Glaser<sup>3</sup>, L.C. Groop<sup>4</sup>, J. Meyer<sup>1</sup>.* 1) Millennium Pharmaceuticals, Inc., Cambridge, MA, USA; 2) Division of Metabolism, Endocrinology and Diabetes, Washington University Medical School, St. Louis, MO, USA; 3) Department of Endocrinology and Metabolism, Hebrew University, Hadassah Medical Center, Jerusalem, Israel; 4) Department of Endocrinology, Wallenberg Laboratory, Malmo University Hospital, University of Lund, Malmo, Sweden.

The scavenger receptor class B type 1 (SR-B1) is involved in the selective transport of lipids from HDL cholesterol. We examined polymorphisms in the SR-B1 gene to determine their association with plasma HDL-C levels. Nested sex-specific case-control studies were designed from families originally ascertained for Type 2 diabetes from Finland, Sweden and Israel. Cases (n=558) and controls (n=379) were chosen based on having HDL-C levels below (cases) and above (controls) the sex-specific median for the combined populations. Genotypes for a silent variant in exon 8 (alleles EX8C and EX8T) and an intron 5 variant (alleles IVS5C and IVS5T) were evaluated. All three populations produced similar results. Carriers of EX8C who lacked the IVS5T allele had increased odds of low HDL-C (odds ratio for combined populations of 2.44,  $p < .0001$  and 1.95,  $p < .01$  for women and men, respectively). In women, a second pattern marked by the presence of both EX8C and IVS5T produced  $>4.7$ -fold increased odds of low HDL-C ( $p < .00001$ ) but no association was found in men. These patterns may represent two distinct haplotypes where the underlying variant on the second haplotype acts in a sex-specific manner. These results demonstrate strong, highly significant and reproducible associations with genetic variants in SR-B1 and HDL-C levels.

Program Nr: 1172 from the 2001 ASHG Annual Meeting

**The Use of the Disposition Model For Genetic Analysis. I. Association, Segregation and Linkage Studies. V.**  
*Apprey, J. Kwagyan, G.E. Bonney.* National Human Genome Ctr, Howard Univ, Washington, DC.

The disposition model of Bonney (1998) for binary phenotypes merges the likelihood models of Elston and Stewart (1971) and Morton and Maclean (1974). Unlike the earlier regressive models, the disposition model does not assume that sibs are arranged according to their birth order, and so they are interchangeable. In this presentation, fuller parametrization are described for both segregation and linkage analyses. The regression parametrization includes gene by environment interactions on the logistic as well as the Weibull curves. The inclusion of ascertainment corrections is discussed and the easy implementation in the computer package G.E.Ms noted.

**Haplotype frequency estimation error analysis via the application of bootstrap methods.** *E.D. Kelly<sup>1</sup>, F. Sievers<sup>1</sup>, O.R. McManus<sup>2</sup>*. 1) Hitachi Dublin Laboratory, Hitachi Europe Limited, Dublin 2, Ireland; 2) Dept. of Clinical Medicine, Trinity College Dublin Centre at St. James's Hospital, Dublin 8, Ireland.

Increasingly researchers are turning to the use of haplotype analysis as a tool in population studies, the investigation of linkage disequilibrium, and candidate gene analysis. When the phase of the data is unknown, computational methods, in particular those employing the Expectation-Maximisation (EM) algorithm, are frequently used for estimating the phase and frequency of the underlying haplotypes. Despite its widespread use, there has been little investigation of the degree of error involved in haplotype frequency estimation (HFE) employing the EM process. We have developed an EM-based program for the estimation of haplotype form and frequency from phase-unknown data which incorporates non-parametric bootstrapping for the calculation of accurate error bars. To illustrate its efficacy we present the results when HFE is carried out on data derived from a population of cystic fibrosis patients. Alleles from seven biallelic loci are used. Initially the phase of the data is known and the frequencies of the observed haplotypes are calculated by a simple gene counting method. The phase is then randomised and the haplotype frequencies are recalculated using an EM-based algorithm. The technique of bootstrapping is then applied to generate error bars. The accuracy of the EM method and the sensitivity of the bootstrap procedure to data sizes are assessed via a comparison between the results for the phase-known and the phase-unknown data. It was found that for large data sets the estimation process reproduced the original haplotypes and their frequencies from the randomised data with a high degree of accuracy. As expected, the accuracy of the estimation falls off significantly as less individuals are included in the analysis.

Program Nr: 1174 from the 2001 ASHG Annual Meeting

**Power of Multifactor Dimensionality Reduction (MDR) for Identifying Gene-Gene and Gene-Environment Interactions.** *L.W. Hahn, M.D. Ritchie, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

We have previously developed the multifactor dimensionality reduction (MDR) method to identify gene-gene and gene-environment interactions (Ritchie et al. *AJHG* 69, 2001). In brief, MDR is a method that reduces the dimensionality of multilocus information to identify polymorphisms associated with an increased risk of disease. This approach takes multilocus genotypes and develops a model for defining disease risk by pooling high-risk genotype combinations into one group and low-risk combinations into another group. Ten-fold cross validation and permutation testing are used to identify optimal models. The goal of this study was to evaluate the power of MDR for identifying gene-gene and gene-environment interactions in the presence of common sources of noise. Using four different epistasis models, we simulated discordant sib-pairs with 5% genotyping error, 5% phenocopy, 20% phenocopy, or 50% genetic heterogeneity. MDR was able to identify the functional loci with 81-99% power in the presence of genotyping error or phenocopy, and 47-81% power in the presence of genetic heterogeneity. These results demonstrate that MDR is a powerful method for identifying and characterizing gene-gene and gene-environment interactions, even in the presence of some common sources of noise.

**Identifying extreme regions of linkage disequilibrium with dense maps.** *R. Mott, G.R. Abecasis, L.R. Cardon.*  
Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom.

Large compilations of single nucleotide polymorphisms (SNPs) are now available, and significant advances are underway in genotyping throughput, accuracy, and cost. Together, progress in these areas offers great promise for studies of allelic association on a genomic scale. Despite these advancements, it is generally impossible to systematically evaluate long-range haplotype patterns in large datasets of pair-wise linkage disequilibrium (LD). One of the key issues to resolve is the identification and characterisation of regions with generally excessive or low LD. The statistical theory of extreme-scoring segments, originally developed for sequence alignment problems, provides a natural framework for LD assessment. We show that this approach can be used to identify salient topological features in LD matrices. Moreover, significance tests based on the Gumbel extreme value distribution can be used to critically evaluate extreme patterns of LD. Using these methods, we identified excessively long stretches of high and low disequilibrium in the evaluate extreme patterns of LD. Using these methods, we identified excessively long stretches of high and low disequilibrium in the chromosome 22 disequilibrium map, which includes over 1 million pair-wise LD coefficients for 1500 markers. These regions may be the signature of selection intensity or reflect functionally important features in DNA.

**Dynamic Models for Mapping Quantitative Trait with Time-Dependent Genetic Effect.** *M. Xiong, J. Zhao, L. Jin, E. Boerwinkle.* Human Gen Ctr/Houston HSC, Univ Texas, Houston, TX.

Many quantitative traits of interests in genetic studies of complex diseases such as hypertension, obesity and diabetes are known to change with age. The genetic effects of these traits are, in general, time dependent. The appropriate models for time-dependent genetic effects are very useful for mapping quantitative trait loci (QTL) and genetic epidemiology study of complex traits. However, very few statistical models for time-dependent genetic effects have been developed. In this report, a linear and nonlinear dynamic system is proposed to model the time-dependent genetic additive and dominance effects. A quantitative trait is modeled as a functional-coefficient regression in which the genetic effects are treated as fixed functions and environments are treated as random functions. The random functions are modeled as realization of Gaussian processes. The functions are represented by support vector machine. The Kalman filter is used to fit the functional models. The proposed dynamic models have been applied to the genetic studies of hypertension using longitudinal data collected from a population.

**Application of Symbolic Discriminant Analysis to Identifying Multivariate Gene Expression Profiles that Define Autoimmune Diseases.** *J.S. Parker, T. Aune, S. Chan, A. Martin, A. Antar, K. Maas, N. Olsen, J.H. Moore.* Program in Human Genetics, Vanderbilt Univ, Nashville, TN.

It is increasingly clear that our ability to simultaneously measure the expression levels of thousands of genes will revolutionize our understanding of the initiation, progression, and severity of human disease. However, progress in this area is dependent on the development and implementation of analytical methods that are able to identify gene expression patterns that are associated with a particular biological or clinical endpoint. We have previously developed a symbolic discriminant analysis (SDA) approach to identifying biologically or clinically relevant gene expression variables (Moore et al. *Lecture Notes in Artificial Intelligence*, in press). Briefly, SDA is a statistical and computational methodology for finding mathematical expressions, in symbolic form, that can take values of gene expression variables and produce biological or clinical endpoint predictions. SDA models are optimized using parallel genetic programming and are evaluated for their ability to classify and predict observations using 10-fold cross-validation. Permutation testing is used to evaluate statistical significance. The goal of this study was to determine whether SDA is capable of identifying subsets of gene expression variables that define two autoimmune diseases, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). We measured the relative expression levels of approximately 4000 genes in a sample of seven RA subjects, nine SLE subjects, and 12 control subjects. The SDA approach identified a subset of four statistically significant genes that were capable of correctly classifying all RA and control subjects and a subset of five statistically significant genes that were capable of correctly classifying all SLE and control subjects. These results demonstrate that SDA is able to automatically identify subsets of autoimmune disease genes and their associated discriminant functions.

**Correlation of Gene Expression Levels with Clinical Covariates in Microarray Experiments.** *W.D. Shannon<sup>1</sup>, M. Watson<sup>2</sup>, A. Perry<sup>2</sup>, K. Rich<sup>3</sup>.* 1) Dept. of Medicine; 2) Dept. of Pathology and Immunology; 3) Dept. of Neurosurgery, Washington Univ School of Medicine, St Louis, MO.

Analyzing microarray data generally starts with a clustering algorithm (e.g., hierarchical cluster analysis, self-organizing maps) to separate genes into subgroups with similar expressions, and then compares the clinical measure of interest across the identified clusters. If the clinical measure, such as tumor grade, is disproportionately present across one or a few clusters, the genes within those clusters may be functionally related to the measure. This "two-stage" analysis is likely to be sub-optimal since the analysis of the gene expressions and clinical measures (i.e., covariates) are performed sequentially. Ideally, a statistician would prefer a single analysis (e.g., correlation or regression) using gene expression and covariate data simultaneously. The Mantel correlation statistic, and its extension to the partial correlation and regression frameworks, allows the simultaneous analysis of expression data and covariates in microarray studies. In this paper, we describe this family of statistics and apply them to a previously published study of seven human oligodendrogliomas (brain tumors) where the expression levels of 1,013 genes and five covariates were analyzed using the "two-stage" approach described above. In the previous analysis, qualitative relationships were found between gene expressions and two of the clinical covariates. In this analysis, the Mantel statistics quantify these relationships, and using permutation tests, provide P values of statistical significance. We also show how the Mantel statistics can be used to rank subsets of genes that are sufficient for tumor grade determination. We propose the use of the Mantel statistics as a valuable contribution to the future of microarray study data analysis.



**Genetic heterogeneity comprising both X-linked and autosomal dominant forms of inheritance in families with familial idiopathic scoliosis.** *C.M. Justice<sup>1</sup>, N.H. Miller<sup>2</sup>, B. Marosy<sup>2</sup>, J. Zhang<sup>3</sup>, E.W. Pugh<sup>4</sup>, A.F. Wilson<sup>1</sup>.* 1) Genometrics Section, NHGRI/NIH, Baltimore, MD; 2) Dept. of Orthopaedic Surgery, Johns Hopkins University, Baltimore, MD; 3) Dept. of Genetics, Johns Hopkins School of Medicine, Baltimore, MD; 4) CIDR, Johns Hopkins University, Baltimore, MD.

Idiopathic scoliosis (IS) is a structural lateral curvature of the spine present in the late juvenile or adolescent period in otherwise normal individuals. It affects 2-3% of the pediatric population, of which 0.2-0.5% require active treatment. Previous studies have suggested autosomal dominant, X-linked and/or multifactorial modes of inheritance. In a large ongoing study of familial scoliosis, 204 families with at least two affected individuals were ascertained and clinically characterized. Phenotypes include degree of lateral curvature, curve type, age of onset and sex. A genome-wide screen for 1200 individuals was performed at the Center for Inherited Disease Research. Since the sample may include both X-linked and autosomal dominant forms of the disorder, the families were stratified on the ratio of the likelihood of each family given an X-linked model relative to that of an autosomal model. The mode of inheritance for families in the upper tail of the distribution was assumed to be X-linked, while families in the lower tail were assumed to be autosomal dominant. Model-dependent and model independent linkage analysis was performed on subsets based on the ranked distribution from both tails of the distribution. Two-point linkage analysis of the top 25% of families in the upper tail of the distribution resulted in positive lod scores for 5 of 8 adjacent markers at a recombination fraction of 0.3. The highest lod score was 1.12 ( $\theta=0.3$ ) for marker CXS1725. Analysis of the top 15% of families resulted in 6 out of 8 adjacent markers positive at a recombination fraction of 0.3. A lod score of 1.85 was obtained for marker CXS1725 ( $\theta=0.2$ ). Genotyping of three additional flanking markers in this area also resulted in positive lod scores. These results support a hypothesis of genetic heterogeneity with a proportion of families having X-linked inheritance.

**Association Between Insulin-Like Growth Factor I (IGF-I) Gene Promoter Polymorphisms and Bone Mineral Density in Elderly Women and Men: The Rotterdam Study.** *F. Rivadeneira<sup>1</sup>, J.J. Houwing-Duistermaat<sup>1</sup>, N. Vaessen<sup>1,2</sup>, A. Hofman<sup>1</sup>, H.A. Pols<sup>1,2</sup>, C.M. van Duijn<sup>1</sup>, A.G. Uitterlinden<sup>1,2</sup>.* 1) Dept. of Epidemiology and Biostatistics, Erasmus University Rotterdam, The Netherlands; 2) Dept. of Internal Medicine, Erasmus University Rotterdam, The Netherlands.

Bone mineral density (BMD), a major determinant of the risk of osteoporosis in later life, is a complex trait influenced by many genes. Conflicting results have been reported, regarding the relation of a dinucleotide-repeat polymorphism in the promoter region of the insulin-like-growth-factor-I (IGF-I) gene to osteoporosis and BMD. In the present study we examined the role of this polymorphism in relation to BMD measurements, using a cross-sectional and follow-up design within the Rotterdam study, a population-based cohort study of determinants of chronic disabling diseases in the elderly. We found the polymorphism to predict the yearly rate of bone loss in 1570 individuals, and not the cross-sectional evaluation of BMD in 2161 individuals. The mean follow-up time between the two BMD measurements used to estimate the yearly rate of BMD change was 23.8 (SD 7.0) months. In women (n=838), the presence of the *192-bp* allele was associated (p for trend = 0.0005) with a lower yearly rate of bone loss (an absolute reduction of 8.8 and 13.2 mg/cm<sup>2</sup> and a relative reduction of 1.5 and 0.5%; for hetero and homozygotes for the *192-bp* allele, respectively). In men (n=732), the genotype effect was only evident (p for trend = 0.05) when taking into account effect modification of age (p=0.06). These findings showed that in elderly Caucasian populations the relationship between this IGF-1 gene promoter polymorphism and rate of BMD loss is different among genders. This is also, the first attempt to evaluate the effect of this polymorphism on BMD with a follow-up approach, permitting to partition the effect of bone mass acquisition during life from the subsequent rate of bone loss, and hence, increasing power. Further evaluation of sequence variation at this locus is needed to determine if the observed effect is a direct consequence of IGF-I mRNA expression or if it is the result of linkage disequilibria with other polymorphisms.

**Cognitive function in neurofibromatosis type 1.** *S. Costabel, A. Iester, M. Bonelli, S. Massa, D. Sambarino, C. Bellini, E. Bonioli.* Pediatrics Department, University, Genova, Italy.

Neurofibromatosis 1 is associated with a broad range of nonspecific cognitive impairment, including low IQ, learning disabilities and behavioral difficulties. Early reports gave marked overestimates of the incidence of mental retardation. Recently many authors have suggested that the frequency of mental retardation (defined as full-scale IQ more than 2 SD below the mean) due to NF1 alone is only slightly higher than in the general population (3%). On the other hand, specific learning disabilities are the most common neurological complication of NF1 in childhood with a frequency of 30-60% of the NF1 patients. In the past many studies have suggested a distinct profile of learning disabilities in children with NF1, with a predominance of visuo-perceptual problems and discrepancy between Verbal and Performance IQ. However, more recent studies have demonstrated that language based learning problems are as common as non-verbal learning deficits. We have evaluated 15 subjects aged 2 to 19 years. All subjects had NF1 according to NIH-criteria. The global IQ was estimated with different and age-related tests: the Brunet-Lezine scale for children below the age of 2 years; the Stanford-Binet Scale for children aged 2 to 10 years; the Weschler Intelligence Scale for Children-Revised (WISC-R) for children aged 10 to 14 years; the Weschler Adult Intelligence Scale (WAIS) in the older patients. Motor abilities were assessed in two areas: visual-motor function using the Raven's Progressive Matrices and perceptual-motor function using the Bender-Gestalt test, which is suitable in the children above 4 years. We have observed a normal full-scale IQ in our patients, with a mild lowering especially in the young ones. We have also observed abnormal results in Bender- Gestalt test, with a greater than 2 years discrepancy between chronological and test-estimated age. On behavioral and neuropsychological assessments we have observed the presence of attentional and organisational deficits; speech problems were observed especially in the younger patients, in whom we have also observed expressive language disorders.

**DIFFERENT ETIOLOGY IN FAMILIAL LOW-GRADE GLIOMA AND HIGH-GRADE GLIOMA ?** *B.S. Malmer, R. Henriksson, H. Gronberg.* Dep of Radiation Sciences, Onc, UME University Hospital, UME, SwedenÅÅ.

**BACKGROUND:** Familial glioma occurs in about 5% of all glioma patients. Only in a few cases cancer-prone syndromes as the Li-Fraumeni syndrome are known in these families. To assess the mode of inheritance and the risk for low-grade and high-grade glioma respectively, we conducted a segregation analyses and a nation-wide cohort study. **METHODS:** We Constructed two cohorts. Firstly, one of first-degree relatives (FDR) to glioma patients diagnosed 1985-1993 in Northern Sweden. A segregation analyses was performed of the 2141 FDR. A second cohort was then constructed from the Swedish Family-data base with all FDR born 1932 -1997 to patients with low-grade glioma (N=15 321 FDR) and high-grade glioma (N =26 635 FDR) diagnosed 1958 to 1997 in all of Sweden. The risk for all FDR and the risk for siblings were calculated using the program PYRS, calculating person-years 1958-1997. The expected rates of low-grade glioma and high-grade glioma were calculated from the population respectively. **RESULTS:** Segregation analyses: The segregation analyses rejected the sporadic model and an autosomal recessive gene provided the best fit. Family cohort study: A increased risk was shown among FDR of low-grade glioma patients (SIR 3.65, 95% C.I 2.31-5.47), and the risk was higher among FDR to probands younger than 40 years (SIR 4.75 95% C.I 2.86-7.42). However, the risk was even higher in the cohort of siblings (SIR 7.00 95% C.I 3.35-12.87). ( Skall du inte skriva ngot om HGG families, Br du inte ocks skriva ngot om risken fr HGG hos LGG probander fr att belgga din hypotes?) In the low-grade glioma families with siblings, there were no other types of cancers within the family. In the high-grade glioma sibling families, there were other types of cancer among the parents to the siblings indicating an aetiology of multifactorial origin or of other cancer-prone syndromes, as the Li-Fraumeni syndrome. **CONCLUSIONS:** This is the first cohort analysis calculating risk for low-grade and high-grade glioma separately. We have identified a new type of familial glioma where an autosomal recessive gene could be involved, especially for siblings in low-grade glioma.

**Familial epilepsy: clinical and epidemiological features in 10 Cuban families.** *C. Llanusa Ruiz*<sup>1</sup>, *R. Garcia*<sup>2</sup>, *L. Paz*<sup>3</sup>.  
1) Genetics Department, Ramon Gonzalez Coro Hospital, Havana City, Cuba; 2) Juan Manuel Marquez Hospital; 3) Facultad Finlay-Albarran.

Epilepsy is considered a complex disease. Genetic and environmental are known to contribute to the etiology of this disorder. Our group is studying familial epilepsy in Cuba, with the goal of delineating its clinical and epidemiological characteristics and eventually identify susceptibility genes. Here we present preliminary data on ten Cuban families with three or more affected individuals with cryptogenic or idiopathic epilepsy and age at onset less than 25 years old, ascertained through index cases from several hospitals and medical centers in Havana City, Cuba. All the affected individuals and close relatives were interviewed. Family history information about relatives status was obtained with two interview methods. Neurological evaluation was performed on every affected family member and the clinical history was reviewed. The most frequent type of epilepsy found was partial, and in some families there were individuals with partial and generalized epilepsy. Empiric recurrence risks were assessed in each family.

**Screening of the HOXA1 and HOXB1 genes in a group of autism subjects.** *Z. Talebizadeh<sup>1</sup>, D.C. Bittel<sup>1</sup>, J.H. Miles<sup>2</sup>, M.G. Butler<sup>1</sup>.* 1) Children's Mercy Hospital and University of Missouri-Kansas City School of Medicine, Kansas City, MO; 2) University of Missouri School of Medicine, Columbia, MO.

HOX genes are neurodevelopmental genes involved in early brain formation. Allelic variations have been reported in the human HOXA1 (at 7p) and HOXB1 (at 17q) genes including a single base substitution (A218G) in a series of histidine repeats and a 9-base insertion after base 88, respectively. Recent evidence from a study of 57 autistic probands (Ingram et al., 2000) suggested a role for the HOX genes in the susceptibility to autism. To further investigate whether allelic variations of the HOXA1 and HOXB1 genes are over represented in autistic subjects, we studied 40 autistic probands using automated direct DNA sequencing. Both allelic variants of each gene were observed among the studied population. The frequency of the different alleles in these two HOX genes were compared between our autism population and those subjects reported previously by Ingram et al. (2000). Confidence intervals were used to determine if a difference between the two proportions existed. For the HOXA1 gene, our autism population had 26% A/G genotype frequency and Ingram et al. found 38% A/G among their autistic probands. This difference was not statistically significant (95% CI: -30% to 8%). In addition, no significant difference was observed between the A/G genotype frequency in our autism population compared with the controls (22%) reported by Ingram et al. (95% CI: -11% to 20%). For the HOXB1 gene, a statistically lower rate of the insertion genotype (+/Ins) was seen in our autism population (25%) compared to the autistic probands (47%) reported by Ingram et al. (95% CI: -42% to -2%). Comparison of the frequency of this genotype in our autistic probands (25%) with their control population (37%) did not indicate any significant difference (95% CI: -29% to 6%). In addition, we found no evidence of an interaction between the HOXA1 and HOXB1 genes. The results of the current study do not support an association between these HOX genes and autism. Therefore, additional studies with autistic subjects should be performed to clarify whether there is any association between the HOX genes and autism.

**MECP2 mutation type, affected domain, X-inactivation and phenotypic outcome in Rett syndrome. L.S.**

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Rett Syndrome (RTT) is a severe neurodevelopmental disorder primarily affecting females and exhibiting marked clinical variability. It is caused by mutations in the dominant X-linked MECP2 (Methyl-CpG-binding protein 2) gene. We have analysed the clinical variability in RTT by correlating the type of MECP2 mutation present, domain affected and degree of skewing of X-inactivation with the phenotype. The coding region of MECP2 was completely sequenced in 81 patients, 67% of whom had mutations. The other patients may have mutations within the 5' or 3' UTRs or other controlling elements, or may represent phenocopies. Symptoms were assigned severity scores that could be tallied to give an overall phenotypic picture. Truncation mutations were associated with more severe phenotypes, as were mutations affecting the methyl-binding domain (MBD). X-inactivation was examined by a methylation assay of the androgen receptor microsatellite. Skewed X-inactivation (>75% expression of a single allele) was found in 43% of 72 patients, which is higher than that seen in a normal population (16.4%, Busque et al 1996). However, there was no relationship between skewed X-inactivation and any of the phenotypic variables, probably because it may be either protective or detrimental. Nevertheless, it is probable that the variant phenotypes seen in RTT are the result of complex interactions between mutation type, domain affected and X-inactivation.

**Type 2 diabetes, APOE gene and the risk for dementia and dementia related pathologies. The Honolulu-Asia Aging Study.** *R. Peila<sup>1</sup>, B.L. Rodriguez<sup>2</sup>, L.J. Launer<sup>1</sup>*. 1) Laboratory of Epidemiology, Demography and Biometry, NIA, NIH, Bethesda, MD, USA; 2) University of Hawaii at Manoa, Honolulu, HI, USA.

Background: Type 2 diabetes has been implicated as a risk factor for dementia but its interaction with genetic risk factors and the associated pathological mechanisms remains unclear. Objective: To determine the association of diabetes alone or in combination with the APOE gene to incident dementia and to examine the combined effect of APOE genotype and diabetes to neuropathological outcomes in the brain. Methods: Data were collected prospectively from a population-based cohort of 2574 Japanese-American men enrolled in the Honolulu-Asia Aging Study (HAAS). Diabetes was ascertained by interview and direct glucose testing (fasting and 2hr) and APOE genotype was characterized. Dementia was assessed in 1991 and in 1994 by clinical examination, MRI and diagnosis according to international guidelines. Complete autopsy information was available on a representative sample of 216 men. Results: After 2.9 years of follow-up, 135 dementia cases were diagnosed; 51 with Alzheimer's disease (AD) without cerebrovascular disease, 25 with AD and cerebrovascular disease, 33 with vascular dementia (VsD) and 28 with other types of dementia. Diabetes was associated with dementia (relative risk [RR], 1.5 [95% CI 1.01-2.2] for total dementia, 1.7 [CI 1.1-2.9] for AD, and 2.4 [CI 1.1-5.3] for VsD). Individuals with both diabetes and e4 allele had a RR of 5.4 (CI 2.1-13.4) for AD compared to those without AD and the e4 allele. In the autopsy sub-group, diabetes was associated with brain infarcts equal or greater than 1 cm and neuritic plaques (NP) (incidence rate ratio [IRR], 1.8 [CI 1.2-2.6]) and IRR, 1.5 [CI 1.1-2.1]). The group with diabetes and the e4 allele had a higher number of NP and neurofibrillary tangles in the hippocampus (IRR, 4.0 [CI 2.2-5.2] and IRR, 2.8 [CI 2.5-3.2]) and higher risk of cerebral amyloid angiopathy (RR 6.5 [CI 1.4-29.6]). Conclusions: Type 2 diabetes is a risk factor for VsD and AD. The association between diabetes and Alzheimer is particularly strong among carriers of the APOE e4 allele. The results on clinical outcome are supported by the pathological data.



**A Genetic Hypothesis for Chiari Type 1 Malformation with or without Syringomyelia.** *M.C. Speer<sup>1</sup>, D.S. Enterline<sup>2</sup>, T.M. George<sup>3</sup>, A. Frankin<sup>1</sup>, L. Mehlretter<sup>1</sup>, C.M. Wolpert<sup>1</sup>, T.H. Milhorat<sup>4</sup>.* 1) Medicine, Duke Univ Medical Ctr, Durham, NC; 2) Radiology, Duke Univ Medical Ctr, Durham, NC; 3) Surgery, Duke Univ Medical Ctr, Durham, NC; 4) Neurosurgery, State University of NY, New York.

The Chiari 1 malformation (CM1) is defined as herniation of the cerebellar tonsils through the foramen magnum and has a prevalence of approximately 1/5000. CM1 is the leading cause of syringomyelia (S). Recently, we and others have proposed a volumetrically "too small" posterior fossa as causative. We propose a genetic hypothesis to at least a subset of CM1/S cases based on familial clustering in a rare disease, co-segregation with known genetic syndromes, and concordance in like-sex twins. We have identified 67 multiplex pedigrees. Some families demonstrate patterns consistent with male-to-male transmission and transmission across more than two generations. Additional support for a genetic hypothesis for CM1/S comes from co-segregation of CM1 with known genetic syndromes including, among others, achondroplasia, Klippel Feil sequence, Hadju-Cheney syndrome, Albright hereditary osteodystrophy (pseudohypoparathyroidism) and hypophosphatemic rickets, most of which involve an abnormality in bone development. Lastly, we identified 8 sets of like-sex twins in our family ascertainment efforts in CM1/S (7 female; 1 male); 7 of 8 are concordant for CM1 (one female twin pair is discordant for CM1). 3 are concordant for associated syringomyelia. Determinations of the range of  $I_s$  are at least 10 across a broad range of prevalence estimates and recurrence risk to siblings. These data are consistent with a genetic hypothesis in at least subset of CM1/S. We hypothesize that the underlying gene or genes for CM1/S will have pleiotropic effects influencing the extent of tonsillar herniation, posterior fossa volume, and/or other variables such as bony abnormalities in the base of the skull and/or syringomyelia.

**Distribution of the GAA repeat of frataxin gene among the Japanese ataxia patients.** *M. Watanabe, X. Zhaoh, S. Nissato, S. Shoji.* Neurology/Inst Clinical Sci, Tsukuba Univ, Tsukuba, Ibaraki, Japan.

To estimate the relative frequency of Friedreich ataxia in Japanese population, we analyzed the GAA repeat of the frataxin gene among the Japanese ataxia patients. Twentyseven familial ataxia patients and seven sporadic ataxia patients were analyzed. No expanded allele was detected. All patients were either homozygous or heterozygous for normal alleles. The smallest allele was 2 repeats and the largest was 7 repeats. All alleles detected were within short normal allele (SN) range that was reported to be up to 10 repeats. In normal European populations, the GAA repeats of the frataxin gene were reported to show a bimodal distribution that was composed of short normal allele (SN) with 5 to 10 GAA triplets and long normal allele (LN) with 12 to 60 triplets. Frequencies of alleles were compared between reported European population and our samples by Yates corrected  $\chi^2$  test of homogeneity. There was a significant difference between these samples ( $\chi^2$  value was 9.27).

**Canadian Collaborative Study of twins with multiple sclerosis.** *C.J. Willer<sup>1</sup>, R. Holmes<sup>2</sup>, I. Yee<sup>2</sup>, N. Risch<sup>3</sup>, A.D. Sadovnick<sup>2</sup>, G.C. Ebers<sup>4</sup> and Canadian Collaborative Study Group.* 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Department of Genetics, Stanford University, Palo Alto, CA; 4) Department of Clinical Neurology, University of Oxford, Oxford, UK.

The Canadian Collaborative Study on the Genetic Susceptibility to Multiple Sclerosis (CCPGSMS) is a multi-centre study involving 18 MS clinics across Canada and has ascertained over 18,000 individuals with multiple sclerosis (MS). Every individual identified was asked if they had a twin or triplet. 380 Canadian twin probands and 6 Canadian triplet probands were ascertained in this manner, comprising a total of 368 twin pairs and 6 triplet sets.

Twin pairs lost to follow-up (N=15) and pairs in which the co-twin died at birth (N=30) or before the age of 20 (N=1) were excluded from analyses, such that concordance and zygosity data were available for 322 pairs and 334 probands. There were 125 monozygotic (MZ) and 197 dizygotic (DZ) twin pairs identified. At least one twin had been diagnosed with multiple sclerosis using the standard criteria. The unaffected co-twin was examined whenever possible by a neurologist specializing in MS. Based upon the known twinning rate, the population of Canada and the prevalence of multiple sclerosis, it was estimated that between 350 and 450 twin pairs and 4-7 triplet sets with multiple sclerosis would be identified.

The pairwise concordance rate in MZ pairs was  $20.0 \pm 3.6\%$  (25/125), which was significantly higher than the observed concordance rate in DZ pairs of  $5.6 \pm 1.6\%$  (11/197,  $z = 3.77$ ,  $p < 10^{-3}$ ). Probandwise concordance rates were  $25.9 \pm 3.8$  for MZ twins and  $6.5 \pm 1.8$  for DZ twins ( $z = 4.7$ ,  $p < 10^{-5}$ ). The DZ concordance rate was not significantly different than the observed recurrence risk in siblings of the same probands (18/603,  $z = 1.56$ ,  $p > 0.10$ ). This twin series is currently being examined for differences in early life events and for concordance of clinical phenotype. The implications of this study on models of genetic inheritance of multiple sclerosis are discussed.

**The rate of multiple sclerosis (MS) in relatives of MS patients in the province of Sassari, Sardinia.** *M. Pugliatti<sup>1</sup>, A. Sotgiu<sup>1</sup>, S. Sotgiu<sup>1</sup>, A. Sanna<sup>1</sup>, I.M.L. Yee<sup>2</sup>, G. Rosati<sup>1</sup>, A.D. Sadovnick<sup>2</sup>*. 1) Inst. of Clinical Neurology, University of Sassari, Sassari, Italy; 2) Dept. of Medical Genetics, University of British Columbia, Vancouver, Canada.

Recent epidemiological data show that Sardinians are at very high risk for MS. Geographic isolation and consistent inbreeding rates over time in this population lead to the hypothesis of a relatively greater role for genetic susceptibility in MS compared with other populations. The aim of this study is to quantify the rate of MS in relatives of patients living in the province of Sassari.

Preliminary data are presented on 174 unrelated individuals ("index cases") who were (i) independently ascertained, (ii) of clear Sardinian ancestry based on 4 grandparents, and (iii) fulfilling the Poser et al. Committee diagnostic criteria for definite MS. The gender ratio in the study group was 2.5.

The average age (SD) of the index cases was  $36.0 \pm 9.4$  years. Clinical information was obtained for a total of 6,105 biological relatives of the index cases (1,074 first-, 2,051 second- and 2,980 third-degree relatives). In 25/174 (14.4%) families, at least one person other than the index case had MS. Of these, 6 families (24%) had 2 additionally affected family members and 2 families (8%) had at least 3 additionally affected individuals.

Thirty-five out of 6,105 (0.57%) relatives had been diagnosed as having MS. Among first-degree relatives, 3/326 parents, 14/584 siblings, 1/164 children had MS. Among second-degree relatives, 1/679 nephews and nieces, 2/661 paternal, 1/711 maternal uncles/aunts were affected. Among third-degree relatives, 5/1,473 paternal and 8/1,507 maternal first cousins had MS. These numbers represent crude rates but age-adjusted rates will also be presented.

These findings represent preliminary data of a longitudinal study aimed to give a better comprehension of the role of genetic factors in MS susceptibility among Sardinians living in different provinces.

*Supported by ISS, Rome, FISM, Genoa, grant n. 97/R/69, MURST, Rome and Regione Autonoma della Sardegna..*

**Associations between gene polymorphisms and Alzheimers disease using a large case control study.** *S. Tang<sup>1</sup>, Z. Yamagata<sup>1</sup>, Y. Shindo<sup>1</sup>, Y. Takeda<sup>1</sup>, T. Mizutani<sup>1</sup>, T. Asada<sup>2</sup>.* 1) Health Sciences, Yamanashi Medical University, Japan; 2) Department of Psychiatry, Tsukuba University, Japan.

The subjects were 805 Japanese including 407 patients (168 men and 239 women) with a diagnosis of probable AD based on the NINCDS-ADRDA criteria and 398 spouses (162 men and 236 women) of the subjects served as a control. We analyzed 4 kinds of gene polymorphisms: Apolipoprotein E gene (APOE: e2, e3, e4), Very Low Density Lipoprotein gene (VLDL-R: CGG repeat of exon 3), Interleukin 1 alpha gene (IL1A: a C to T transition in the 5-flanking regulatory region at 889 of the gene) and alpha 1 antichymotrypsin gene (AACT: a microsatellite region consisting of a variable number of dinucleotide repeats, (CT)<sub>n</sub>(AT)<sub>n</sub>). APOE and IL1A gene polymorphisms were determined by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The triplet repeat polymorphisms of VLDL-R and AACT were genotyped by the PCR fragment analysis software of the Long Read TowerV sequencer. The estimating odds ratios (OR) and the multiple logistic regression analysis were performed by SAS statistic analysis package to evaluate the association. APOE: The OR was 2.98 (95% CI=2.51-3.40) for the e4 allele. VLDL-R: The OR for the 5-repeat allele was 1.56 (95% CI 1.32-1.78). IL1A: There was no association between the IL1A (-889) polymorphism and AD. AACT: The frequency of the A10 allele was higher in the AD than in the control but this was not significant. The frequency of the A10 allele significantly increased in the AD patients who did not have APOE e4. The multiple logistic regression analysis indicated that APOE and VLDL-R were significant risks for the development of AD.

**Familial aggregation of second cancers in a childhood sarcoma cohort.** *S. Hwang*<sup>1</sup>, *G. Lozano*<sup>1</sup>, *C. Amos*<sup>2</sup>, *L.C. Strong*<sup>1</sup>. 1) Dept Molecular Genetics, Univ TX MD Anderson Cancer Ctr, Houston, TX; 2) Dept Epidemiology, Univ TX MD Anderson Cancer Center, Houston, TX.

In a childhood cancer cohort designed to characterize familial cancer aggregation, we have observed excess cancer risk in the extended families (kindreds). Increase risk for all types of cancer was characterized by female gender, carrying germline p53 mutation, and younger birth cohort. Excess second and multiple cancers was observed in the cancer-prone families. Here we applied generalized linear models to characterize the second cancer risk aim to better illustrate the cancer phenotype for kindreds with or without germline p53 mutation. We estimated the extent of familial aggregation for second cancer in the cohort under the assumption of linear relationship of cancer risk among the family members. Results of the generalized linear model incorporated generalized estimation equation to adjust for correlation among family members showed a log odds of 0.8 (95% confidence interval: 0.2, 1.4,  $p < 0.01$ ) increase in second cancer risk associated with other family member's second cancer incidence. Germline p53 mutation carriers though had shorter time period between the first and the second cancer diagnoses, the difference was not statistically significant. There was no gender difference in second or multiple cancer risk while birth cohort plays a significant role in individual's risk of multiple cancers.

**Cancer Incidence in BRCA1 Mutation Carriers.** *D.J. Thompson, D.F. Easton and Breast Cancer Linkage Consortium.* CRC Genetic Epidemiology Unit, University of Cambridge, UK.

A number of reports have suggested that the role of BRCA1 in cancer susceptibility is not restricted to the increases in breast and ovarian cancer risk with which it is most commonly associated.

To evaluate the risks of other cancers in BRCA1 mutations carriers we conducted a cohort study of 8121 individuals from 699 families segregating a BRCA1 mutation, ascertained in 30 centres across Europe and North America. Over a quarter of the individuals were known to carry a BRCA1 mutation, and untested family members were weighted according to their estimated probability of being a mutation carrier. Cancer incidence in members of families segregating BRCA1 mutations was compared with expected incidence based on population cancer rates.

BRCA1 carriers were at significantly increased risk of pancreatic cancer (RR = 2.3, 95% CI = 1.3 - 4.1) and of cancer of the uterus (RR = 2.7, 95% CI = 1.7 - 4.2). The uterine cancer excess is not related to tamoxifen use, as it did not decrease when women who could have been prescribed the drug in response to a previous breast cancer were excluded. There was some evidence of an elevated risk of prostate cancer in mutation carriers below 65 years of age (RR = 1.8, 95% CI = 1.0 - 3.3). Seven cases of cancer of the fallopian tube were observed (RR = 49.9, 95% CI = 22.5 - 110.9), and one case of retinoblastoma. Overall, the increased risk of cancer at all sites other than the breast or ovary was small (RR = 1.2, 95% CI = 1.1 - 1.4). These results contrast with those for BRCA2 mutations, which are associated with a larger increased risk of cancers of other types.

**Multiple primary malignancies: A predictor for hereditary cancer syndromes.** *F. Wiklund, H. Grönberg.*

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**BACKGROUND:** Early onset and occurrence of multiple primary malignancies are two common characteristics of hereditary cancer.

**AIMS:** To identify patterns of different tumors which might be of interest to identify new hereditary cancer syndromes.

**METHODS:** Between 1.1.1958 and 31.12.2000 a total of 58747 women in the northern part of Sweden were reported to the regional cancer registry. Among these women 689 (1.2%) had a minimum of two malignancies, of which the first cancer was diagnosed before 50 years of age, and 185 (0.3%) women had at least two breast cancers of which the first breast cancer was diagnosed before 50 years of age. These women were followed from date of the second cancer until death or 31.12.2000.

**RESULTS:** Among women with at least two cancers 73 (10.6%) developed a third cancer. The standardized incidence ratio (SIR) of occurrence of a third cancer was 2.3 (95% CI = 1.8-2.9), with a cumulative probability of third cancer occurrence of 40% (95% CI = 28-49%) at 22 years after diagnosis of a second cancer. Among the 185 women with early onset bilateral breast cancer patients 15 subsequent cancers were observed among 13 women. Significantly elevated SIRs was observed for colorectal cancer (SIR = 5.2, 95% CI = 1.7-25.5) and ovarian cancer (SIR = 7.8, 95% CI = 2.1-19.9). An indication of a higher risk for gastric cancer was also observed (SIR = 7.6, 95% CI = (0.9-27.3)).

**CONCLUSIONS:** Cohorts of early onset multiple primary cancer cases has proved to be useful for analysis of new hereditary cancer syndromes. Among women with a minimum of two early onset cancers a cumulative probability of third cancer of 40% was observed. In this pilot study significantly increased risks for colorectal and ovarian cancer was observed among early onset bilateral breast cancer patients. The increased risk for ovarian cancer is well known but the combination of bilateral breast cancer and colorectal cancer is novel. Results from an extended nationwide study, including over 80000 patients with multiple malignancies, will be presented.



**THE OHIO AMISH POPULATION STUDY: PHASE I.** *S.G. Roth, C. Baldia, K. Archer, A. Bowman, S. Call, R. Rau, I. Comeras, J.A. Westman.* The Ohio State University, Comprehensive Cancer Center, Columbus, OH.

The aim of this study is to identify the occurrence and trends of cancers within the Ohio Amish community, a unique founder population in central and northeastern Ohio. Ohio has the largest Amish population of any state and the largest contiguous settlement of Amish in the world, located mostly in Holmes and surrounding counties. The total Amish population eligible for this study was approximately 24,400 living individuals. The *1996 Ohio Amish Directory*, a listing of 4512 Amish households, was used to select 130 Amish households in Holmes, Wayne, Tuscarawas, and Coshocton counties for contact. Households were selected using a simple randomized sampling protocol. To achieve a 10% precision level, 86 households were required to participate in the study. Each selected household was visited by a research assistant from June to December, 2000 and asked to participate through providing a family medical history of the husband and wife's birth families. There were 92 households accrued (71% accrual rate). Four-generation family health histories were obtained including information regarding cancer diagnoses. The average number of individuals, living and deceased, included in each household's history was 284 (total 26,128). Significant overlap of individuals occurs among household histories due to interrelatedness. A relational database is used for in-depth analysis. Confirmation of reported cancer diagnoses is done through medical records and death certificates. No histories represent known hereditary cancer susceptibility syndromes. However, there is an increased incidence of hematological malignancies in at least two extended families. The 1996 data from the Ohio Cancer Incidence Surveillance System is now available for comparison to the data collected through the randomized study. Here, we present a preliminary analysis of cancer occurrence in the Ohio Amish community.

**APOLIPOPROTEIN E POLYMORPHISM IN ANGIOGRAPHICALLY VERIFIED PATIENTS FROM THE ARGENTINIAN POPULATION.** *V.V.G. Bañares<sup>1,2</sup>, G. Peterson<sup>1</sup>, M. , Espeche<sup>1</sup>, G. Barach<sup>1</sup>, R. Gulayin<sup>3</sup>, E. Sisu<sup>3</sup>, M.E. , Ruiz<sup>4</sup>, E. Bravis Lopez<sup>4</sup>, O.H. Pivetta<sup>2</sup>, M.J. Tavella<sup>1,5</sup>.* 1) Programa de Prevencion del Infarto en la Argentina, Universidad Nacional de La Plata,; 2) Centro Nacional de Genética Médica, ANLIS Carlos Malbrán; 3) Servicio de Hemodinamia,; 4) Instituto de Investigaciones Bioquímicas de La Plata, Argentina. Laboratorio Central, Instituto del Torax, La Plata, en la Argentina, Universidad Nacional de La Plata,; 5) Instituto de Investigaciones Bioquímicas de La Plata, Argentina.

Several studies have indicated an association between the e4 allele of the apolipoprotein E and coronary heart disease in some populations. We determined the apo E genotype as described Hixson y col. in 185 samples, 123 belonging to patients with atherosclerotic injury (L) and 62 without atherosclerotic injury (NL) both facts verified by angiography and under-61-year-old. The chi<sup>2</sup> test showed no significant differences when we compared the apo E e4 allele distribution between L vs. NL. Since the n of our control group was considerably small, we compared the allele distribution between our general population (previously reported) and the L group. The chi<sup>2</sup> test shown significant differences (p=0.015). We conclude that these data suggest that the e4 allele could be one factor contributing to increase the susceptibility to coronary heart disease in our population.

**Association between genetic variation of the dopamine D1 receptor gene and essential hypertension in the Korean population.** *J. Be<sup>1</sup>, K.-T. Kim<sup>1</sup>, B.-Y. Kang<sup>1</sup>, C.-C. Lee<sup>2</sup>*. 1) Seoul Bioscience Institute, Seoul, Gangdong-Gu, Korea; 2) Seoul National University.

Essential hypertension is a multifactorial disease, and has been shown to be associated with dopamine D1 receptor (DRD1) gene. The dopamine D1 receptor (DRD1) known to increase sodium excretion by inhibiting Na-H exchanger and Na,K-ATPase activity. The relationship between the genetic variation of the DRD1 gene and essential hypertension in Korean population was investigated by the DdeI restriction fragment length polymorphism (RFLP) pattern of this gene. There were no significant differences between normotensives and essential hypertensives in allele and genotype frequencies. However, the genotype of DRD1 gene was significantly associated with conventional cardiovascular risk factor such as plasma total cholesterol and LDL-cholesterol levels in normotensives ( $P < 0.05$ ). Therefore, our result suggests that Dde I RFLP of DRD1 gene may be useful as genetic marker in the pathogenesis of cardiovascular disease in Korean population.

**Association of the *Taq1* cholesteryl ester transfer protein (CETP) gene polymorphism with high-density lipoprotein cholesterol and coronary artery disease (CAD) in Asian populations.** C.K. Heng<sup>1</sup>, H.Z. Li<sup>2</sup>, H.Y. Yang<sup>2</sup>, Y.S. Tan<sup>3</sup>, M.C. Tong<sup>3</sup>, N. Saha<sup>1</sup>, P.S. Low<sup>1</sup>. 1) Dept Pediatrics, National Univ Singapore, Singapore; 2) Dept Biochemistry, National Univ Singapore, Singapore; 3) National Heart Centre, Singapore.

We genotyped the CETP *Taq1* polymorphism in the Chinese, Malays and Asian Indians in Singapore and determined its impact on plasma lipid profiles and CAD. Angiographically confirmed CAD patients ( $n=623$ ) who were admitted consecutively for by-pass graft were included as cases. The healthy control subjects ( $n=929$ ) were selected from those attending routine medical examinations. They were free of heart ailments, diabetes, hypertension and stroke.

In the healthy populations, the *B1* allele frequency was highest in the Malays (0.59), intermediate in the Chinese (0.53), and lowest in the Indians (0.51). The Malay *B1* frequency was significantly higher than Indians and the Chinese ( $P < 0.05$ ). The genotype distributions were all consistent with Hardy Weinberg expectations.

A significantly lower *B2* allele frequency was observed in the Chinese CAD patients ( $P < 0.01$ ) compared to the healthy controls. The odds ratio for CAD associated with the absence of *B2* was 3.45 (95% CI 1.69 to 7.39) after adjustment for the confounding effect of cigarette smoking and BMI by logistic regression. The association of the genotypes with high density lipoprotein cholesterol (HDL) levels was significant for male Chinese ( $P = 0.007$ ) and female Indians (0.039) in the order  $B1B1 < B1B2 < B2B2$  and could explain about 5% of the total variation in HDL levels in these two ethnic groups. All analyses were carried out with adjustment for the confounding effects of age, BMI and smoking status. The effect of the *Taq1* polymorphism on apolipoprotein A-1 (apoA1) was similar to HDL, with the *B2* allele associated with elevated apoA1 levels in the Chinese males ( $P=0.001$ ) but weakly in the Indian females ( $P=0.07$ ). We did not find any effect of gene-environmental interaction between CETP genotypes and smoking on plasma lipid levels.

**A Multigenic Model of Hypertension.** *S.O. Henderson<sup>1</sup>, P. Bretsky, MS<sup>2</sup>, B.E. Henderson, MD<sup>2</sup>, D. Stram, PhD<sup>2</sup>.* 1) Emergency Medicine, Keck School of Medicine, USC, Los Angeles, CA; 2) Preventive Medicine, Keck School of Medicine, USC, Los Angeles, CA.

Hypertension (HTN) is a major predisposing condition to mortality from stroke, myocardial infarction and renal failure. African-Americans (AA) have excess HTN when compared to other U.S. populations, an excess particularly significant in younger individuals. Latinos, who share many sociocultural characteristics with AA, have a much reduced risk of HTN, and we have previously suggested that genetic, as well as environmental factors, must be important in the predisposition of the AA population to HTN. The Renin-Angiotensin-Aldosterone system is critical to the maintenance of blood pressure. Variants in the Angiotensin-Converting-Enzyme (ACE) and Aldosterone Synthase (CYP11B2) genes have been associated with the risk of HTN. We genotyped AA and Latino members of a large multiethnic cohort (MEC) to determine the frequency of the ACE D/I variant, and the C344T variant in the CYP11B2 gene, and their association with HTN / anti-HTN therapy. **METHODS:** This was an analysis of the frequency of the ACE D/I and CYP11B2 344 C/T polymorphisms in a subset of a MEC. The genotypes of subjects <65 years of age in the cohort were compared to their HTN status. **RESULTS:** A small increase in risk of HTN is seen for subjects with ACE DD in both AA (R.R.=1.46) and Latinos (R.R.=1.23). The D allele is more frequent in AA (58% vs. 48%). There is an increased risk of HTN for CYP11B2 TT in AA (R.R.=1.63), but not for Latinos (R.R.=0.92). In a simple additive model, there is an increasing risk of HTN in AA, with an increasing frequency of the "at risk" ACE D and CYP11B2 T alleles, such that those with two high-risk alleles have a R.R. of 1.57 (95% CI: 0.79, 3.12), with three alleles have a R.R. of 1.74 (95% CI: 0.89, 3.40), and those with four high risk alleles have a R.R. of 2.07 (95% CI: 0.98, 4.39) (p for trend=0.07). No such pattern was observed in Latinos. **CONCLUSION:** The ACE D and CYP11B2 344T alleles appear to make a modest, and additive contribution to the risk of HTN in AA, but not in Latinos. We are continuing to increase the size of the population under study, as well as the number of candidate gene variants.

**High resolution haplotype analysis of the ACE region in essential hypertension.** *J. Nakura<sup>1</sup>, H. Yamagata<sup>1,2</sup>, A. Morishima<sup>1</sup>, K. Uemura<sup>1</sup>, K. Kohara<sup>1</sup>, T. Miki<sup>1</sup>.* 1) Dept Geriatric Medicine, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept Hygiene, Ehime Univ Sch Medicine, Ehime, Japan.

The D variant in angiotensin-I converting enzyme (ACE) gene is associated with high ACE levels and may be related to increased risk of cardiovascular disease. However, such findings have not been consistent among studies, suggesting that associations between genotypes and diseases may be influenced by additional genetic or non-genetic factors. We previously reported that the ACE polymorphism was associated with cardiovascular disease (*Hum Genet* 107:239,2000) and systolic blood pressure after hospitalization was significantly higher in normotensive male subjects who possessed the D allele (*Hypertens Res* 23:201,2000). To further investigate the influence of the ACE gene region, we genotyped 10 SNPs spanning 26 kb of the ACE gene by ASO method in 100 Japanese hypertensive patients and 100 healthy controls and constructed haplotypes using the computer program HAPLO (*Journal of Heredity* 86:409,995). The haplotype frequency was compared with the data in Caucasian British families. As expected, strong linkage disequilibrium was observed among the SNPs. A total of 38 different haplotypes were observed and two of them accounted for 70%. There was no statistically significant difference in haplotype frequency distributions between cases and controls. However, we found a different frequency distribution pattern of the 9 haplotypes in the Japanese population compared with the distribution pattern previously reported for British families. Moreover, clade C ( recombinant of clade A and B ) did not exist in Japan. These findings may lead to the new consideration of global genetic drift.

**The hepatic lipase gene promoter polymorphism (*LIPC*-480 C>T) is associated with coronary artery calcification in Type 1 Diabetes.** *J.E. Hokanson<sup>1</sup>, S. Cheng<sup>2</sup>, J.K. Snell-Bergeon<sup>1</sup>, M.A. Grow<sup>2</sup>, C. Hung<sup>2</sup>, H.A. Erlich<sup>2</sup>, J. Ehrlich<sup>3</sup>, R.H. Eckel<sup>4</sup>, M. Rewers<sup>1</sup> and CACTI (Coronary Artery Calcification in Type 1 Diabetes).* 1) Preventive Med & Biometrics, Univ Colorado Health Sci Ctr, Denver, CO; 2) Roche Molecular Systems Inc, Alameda, CA; 3) Colorado Heart Imaging, Denver, CO; 4) Dept. of Medicine, Univ Colorado Health Sci Ctr, Denver, CO.

Subjects with Type 1 Diabetes are at increased risk of coronary heart disease (CHD). Coronary artery calcification (CAC) is a measure of subclinical CHD. Hepatic lipase is a rate limiting enzyme in lipid metabolism. We investigated the role of the hepatic lipase gene promoter polymorphism (*LIPC*-480 C>T) and CAC in 91 Caucasian subjects with Type 1 Diabetes.

Among the 48 men and 43 women, the *LIPC*-480 C>T allele frequency was 0.24 and genotypes were in Hardy-Weinberg equilibrium. The *LIPC*-480 T allele was statistically significantly more common in subjects with CAC (see table).

CAC (Au)	n =	<i>LIPC</i> -480 T frequency
0	40	0.14
0.1 - 10	31	0.31
10.1 - 100	22	0.27
> 100	18	0.39

In logistic regression, *LIPC*-480 genotype remained significantly related to CAC, independent of age, sex, duration of diabetes, lipids, and blood pressure.

Thus the hepatic lipase gene promoter polymorphism is associated with the presence of coronary artery calcification in Type 1 Diabetes. If confirmed, the hepatic lipase gene may be an important susceptibility gene for subclinical CHD in Type 1 Diabetes.

**Familial aggregation of cardiovascular disease risk factors in type 2 diabetic families.** *L.A. Lange, L.E. Wagenknecht, C.D. Langefeld, B.I. Freedman, W.A. Riley, S.S. Rich, D.W. Bowden.* Wake Forest University, Winston-Salem, NC.

Atherosclerotic cardiovascular disease (CVD) is a complex disease process with multiple genetic loci likely contributing small individual effects. Targeting a population enriched for type 2 diabetes (T2DM), which is characterized by accelerated CVD, may allow us to detect these effects. An established surrogate measure for CVD is common carotid artery intima-media thickness (CCA-IMT) measured by high resolution B-mode ultrasonography. Other quantitative risk factors for CVD include body mass index (BMI) and lipid measures (HDL, LDL, triglycerides). We hypothesize that these measured CVD risk factors will exhibit a higher degree of heritability in families enriched for T2DM. This sample consisted of 124 individuals (109 diabetics; 109 Caucasians/15 African Americans) from 54 families participating in the Diabetes Heart Study (DHS), a study designed to search for genetic factors contributing to CVD in families enriched for T2DM. The mean ( $\pm$  std) for age was  $60.2 \pm 11.3$  yrs, IMT  $0.65 \pm 0.11$  mm, BMI  $33.2 \pm 8.2$ , HDL  $42.6 \pm 13.9$ , LDL  $116.5 \pm 31.3$  and triglycerides  $192.4 \pm 97$ . Data were analyzed in order to obtain heritability estimates ( $h^2$ ) adjusted for age, gender and ethnicity using the SOLAR software package. BMI and triglycerides were transformed to approximate distributional assumptions. The adjusted  $h^2 \pm$  SE and p-value for IMT ( $0.49 \pm 0.20$ ,  $p < 0.005$ ), square root of BMI ( $0.56 \pm 0.23$ ,  $p < 0.006$ ), HDL ( $0.36 \pm 0.24$ ,  $p < 0.07$ ), LDL ( $0.35 \pm 0.21$ ,  $p < 0.05$ ), and log-triglycerides ( $0.78 \pm 0.21$ ,  $p < 0.0005$ ) all met or were near statistical significance. Since BMI is known to be associated with IMT, we also obtained  $h^2$  for IMT ( $0.52 \pm 0.21$ ,  $p < 0.004$ ) after making an additional adjustment for BMI. Our results support familial aggregation for these CVD risk factors, with IMT, BMI and triglycerides showing the largest heritabilities. These results, taken together with the previous significant  $h^2$  of coronary artery calcium in the DHS population ( $0.50 \pm 0.22$ ,  $p < 0.009$ ; Wagenknecht et al., *Diabetes*, 50:861-866, 2001), suggest that multiple traits are heritable in the DHS sample and that these traits may be important in efforts to map genes contributing to atherosclerotic CVD.



**Genetic basis of variation in carotid artery plaque: The San Antonio Family Heart Study (SAFHS).** *K.J. Hunt<sup>1</sup>, R. Duggirala<sup>1</sup>, H.H.H. Göring<sup>2</sup>, J.T. Williams<sup>2</sup>, L. Almasy<sup>2</sup>, B.D. Mitchell<sup>3</sup>, J. Blangero<sup>2</sup>, D.H. O'Leary<sup>4</sup>, M.P. Stern<sup>1</sup>.* 1) University of Texas Health Science Center, San Antonio, TX; 2) Southwest Foundation for Biomedical Research, San Antonio, TX; 3) University of Maryland School of Medicine, Baltimore, MD; 4) New England Medical Center, Boston, MA.

While many researchers have investigated the genetic basis of carotid artery intima-media thickness, a quantitative marker of sub-clinical atherosclerosis, few studies have focused on the genetic basis of carotid artery plaque (CAP), a discrete marker of sub-clinical atherosclerosis. We investigated the extent to which the presence or absence of CAP was under genetic control in the SAFHS. The study population consisted of 750 individuals distributed across 29 randomly ascertained extended Mexican American pedigrees who participated in the second exam of the SAFHS. Extracranial focal CAP was identified by B-mode ultrasound in the internal and common carotid arteries or the carotid bulb on the right and left side. 51 of 461 women, and 57 of 289 men (aged 18 to 89 years) had evidence of a plaque either in the right and/or left carotid artery. The mean age of participants with and without CAP was 59.2 and 39.3 years, respectively. Using a variance decomposition approach implemented in SOLAR, we performed genetic analysis on the discrete trait CAP (i.e., liability to disease), using a threshold model. Covariates considered in the initial analysis included age, sex, diabetes, current smoking status, and lipid levels, as well as markers of hypertension and obesity. Adjusting for significant age and sex effects only, the heritability ( $h^2 \pm SE$ ) for CAP was significant [ $h^2 = 0.28 \pm 0.15$ , ( $P = 0.01$ )]. Further, after adjusting for additional covariates that contributed significantly to the model ( $P < 0.05$ ; diabetes, systolic and diastolic blood pressure, body mass index, waist circumference, and smoking status) the heritability remained significant [ $h^2 = 0.27 \pm 0.16$ , ( $P = 0.03$ )]. Our results suggest that in the Mexican American population the variation of the discrete trait CAP is under appreciable additive genetic influences after controlling for established cardiovascular risk factors. This work was supported in part by NHLBI grant P01 HL45522.

**Family resemblance for Fat Mass and Fat Free Mass: the Korean Cardiovascular Genome Study.** *Y.S. Jang, E.K. Im, J.H. Kwon, E.Y. Cho, H.Y. Park, S.H. Jee, J.H. Lee.* Dept Cardiology, Yonsei Yonsei Cardiovascular Research institute, Seoul, South Korea.

Obesity is well-known risk factor for cardiovascular disease. The body composition, especially fat mass, is determined by complex phenotype for which multiple genetic and nongenetic factors are involved. Here we applied the regressive model to family data fat mass (FM) and fat free mass (FFM) to investigate the major gene hypothesis in Korean population. The sample consisted of 456 members of 78 families from Korean Cardiovascular Genome Study. Proband was diagnosed hypertension or coronary artery disease. FM and FFM were assessed by bioelectrical impedance (TBF 105, Japan). The data were adjusted for age, age<sup>2</sup>, height, BMI, gender, drinking, exercise, and menopause status prior to analysis. Adjusted factor FM, FFM showed strong familial aggregation with an estimated sibling correlation of 0.27, 0.40 and a smaller parents offspring correlation of 0.13, 0.11 respectively. For FM, the most parsimonious model was a codominant model, and the putative major gene explained the 35.7 % of variance in adjusted FM. The estimated heritability of the FM is 30.4 % by the method of Rice (1997). For FFM, the segregation analysis did not provide statistical evidence of major gene. The differences is partially expressed FFM is more affected by many of environmental factor, kidney function than FM. Our results showed the major gene effect determining the FM in Korean population, and linkage analyses using the genetic markers should be followed for identification of candidate genes.

**The Sca I RFLP of atrial natriuretic peptide gene in Korean essential hypertensives.** *K. Kim<sup>1</sup>, B.-Y. Kang<sup>1</sup>, J.-H. Shin<sup>2</sup>, C.-C. Lee<sup>2</sup>*. 1) Seoulin Bioscience Institute, Seoule, Gangdong-Gu, Korea; 2) Seoul National University.

Essential hypertension is considered to be caused by a complicated combination of genetic and environmental factors. Atrial natriuretic peptide (ANP) has been to suppress renin activity and inhibit the synthesis and release of aldosterone. Therefore, Abnormalities of this peptide caused by genetic variation may be influence the blood pressure. The aim of present study was to examine the relationship between essential hypertension and Sca I RFLP of ANP gene in Korean population. The genotype distribution of this RFLP was significantly different between normotensives and essential hypertensives ( $P < 0.05$ ). However, this genetic marker was not significantly associated with any anthropometric parameters or plasma lipid concentrations in our study group . Therefore, our result suggest that Sca I RFLP of ANP gene may be useful as genetic marker in the ethiology of essential hypertension in Korean population, independent of any cardiovascular risk factors studied.

**Effects of Gene Polymorphisms and Lifestyle Factors on Blood Pressure in a longitudinal study.** *Y. Takeda, Y. Zhang, T. Mizutani, S. Tang, Z. Yamagata.* Health Sciences, Yamanashi Medical University, Tamaho, Yamanashi, Japan.

The purpose of this project was to clarify the relationships among hypertension-related gene polymorphisms, blood pressure and lifestyle factors, as well as their effects on blood pressure changes in a follow-up study of a large Japanese population from 1994 to 1998. The study subjects were healthy unrelated Japanese male employees of a company, aged from 30 to 61 years (mean=42±8.5). Five hundred and thirty subjects were selected at random from among the 1,154 persons. The lifestyles of these 530 subjects were investigated by questionnaire in 1994. A polymerase chain reaction (PCR) analysis was used to detect three hypertension-related gene polymorphisms. The data were analyzed in ANOVA and multiple regression analysis. The mean systolic BP and diastolic BP values were significantly higher in the subjects with the TT genotype of AGT M235T gene polymorphism compared to other genotypes ( $P<0.001$ ). The mean diastolic BP was higher in the subjects carrying the D allele of ACE I/D gene polymorphism, but this difference was not significant. No significant association was observed between AAD gene polymorphism and mean BP. The multiple regression analysis revealed significant correlations of a consistent magnitude between systolic BP and each of the following variables: AGT, family history of hypertension, age, body mass index (BMI) and alcohol consumption ( $P<0.0001$ ). In addition, a significant association between diastolic BP and the ACE gene with adjusted related factors was shown. Smoking was not found to be associated with hypertension in this study population. Our findings suggest that there is an association between AGT gene polymorphism and BP. ACE gene polymorphism had a weak relation to blood pressure. We found no association between AAD gene polymorphism and BP in this Japanese population. The subjects age, BMI, positive family history and alcohol consumption were shown to have strong associations with BP. The results of this study support those of previous epidemiological investigations, except for the absence of a relationship between smoking and BP in this study.

**A Report of Recessive Inheritance in 14 cases with COCKAYNE SYNDROME in 3 families from an Endemic Mexican State, where Genetic Factors (CONSANGUINITY) contribute for the development of the disease. M.B.**

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**INTRODUCTION.** 14 cases of cockayne syndrome (CS) from three different families with first and second degrees of consanguinity living in two small towns in a mexican state of Oaxaca were studied. **CASES REPORT.** The clinical manifestation of CS were analized at the Pediatrical Hospital Para el Nio Poblano (HNP) by the multidisciplinary medical staff. The main clinical manifestations were dermatological alterations as photosensitivity to sunligh and predisposition to skin cancer "xeroderma pigmentosum" (XP); endocrinologic alterations as dwarfism and senil appearance; opthalmological findings from cataracts to pigmentary retinal degeneration; neurological alterations as mental retardation and sensorial hearing loss; and upper limbs, lower limbs and vertebral column degeneration was also found. **CONCLUSIONS.** 14 cases with Cockayne Syndrome were diagnosed in three Mexican families from two different Mexican towns where consanguinity rate is high. By taking in cosideration the 140 cases already reported, we might considere to have the 10% of the general population with Cockayne syndrome, in this state. These results confirmed that consanguinity is, as reported, the etiological factor for this genetical disease. **KEYWORDS:** Cockayne Syndrome; xeroderma pigmentosum; autosomal recessive inheritance.

**Familial Aggregation of Hyperemesis Gravidarum.** *M. Schoenberg-Fejzo*<sup>1</sup>, *L. Anderson*<sup>1</sup>, *R. Schoenberg-Paik*<sup>2</sup>. 1) Department of Medicine, UCLA, Los Angeles, CA; 2) Department of Statistics, UCLA Los Angeles, CA.

Nausea and vomiting in pregnancy, or "morning sickness," occurs in 50-90% of all pregnant women. When the symptoms are so severe that individuals require hospitalization and/or therapeutic intervention, 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 leads to at least a 5% weight loss. Symptoms of HG can include ketonemia, ketonuria, dehydration, electrolyte imbalance, ptyalism, gestational hyperthyroidism, and hepatic and renal damage. Little is known about the etiology of HG, which may be an abnormal toxic response to normal hormone levels during pregnancy, or conversely, may be a normal toxic response to abnormal hormone levels or abnormal metabolites during pregnancy. 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 collected surveys from over 226 affected individuals. Our study shows that the 3 most commonly prescribed antiemetics 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 questions about family history. All patients were diagnosed by a medical professional to have hyperemesis gravidarum. Of the 226 respondents, 74 reported having at least one sister with a pregnancy history. Of the 74 sister pairs, 21 reported a sister with HG. In addition, 24 affected individuals reported having an affected mother, and 27 reported having an affected secondary relative. We have also recruited 13 families with 3 or more affected individuals. 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.

**Twin zygosity and concordance for the autism phenotype.** *A.C. Thomas, K. Osann, C. Modahl, T. Laulhere, M. Smith, J. Escamilla, V. Nguyen, P. Flodman, M.A. Spence.* Human Genetics, Univeristy of California-Irvine, CA.

Autism has been previously demonstrated to have a high heritability. However, no conclusive evidence has been provided regarding phenotypic variation. In this study, ten sets of twins and one set of triplets in which at least one member was diagnosed with Autistic Disorder (AD) were ascertained. Zygosity of the same-sex multiples were determined with greater than 99% accuracy using microsatellite analysis. Subjects were assessed for AD using the Autism Diagnostic Interview (ADI) and the Autism Diagnostic Observation Scale (ADOS). Concordance rates for AD were significantly different between monozygotic (MZ) and dizygotic (DZ) pairs, 100% and 29%, respectively ( $p = .02$ ), indicating a strong genetic component in the development of AD.

Manifestations of autistic behaviors as measured by the ADI and ADOS were compared within concordant MZ pairs ( $n=6$ ), concordant multiples ( $n=8$ , includes both MZ and DZ pairs), and unrelated pairs concordant for AD ( $n=19$ ). Phenotypic variation within the autistic triad was not significantly different for MZ and DZ pairs. Unrelated individuals with AD exhibited greater variation (not statistically significant).

IQ was assessed using the Stanford-Binet Intelligence Scales or the Mullen Scales of Early Learning. MZ pairs concordant for AD were significantly correlated for overall IQ and nonverbal IQ ( $r = 0.94$ ,  $p = 0.003$  and  $r = 0.97$ ,  $p = 0.001$ , respectively). The within-pair correlation coefficient for verbal IQ was moderately high ( $r = 0.70$ ,  $p = 0.094$ ), though not significant. No correlation for IQ was found within DZ pairs and unrelated pairs concordant for AD.

The stronger correlation for IQ observed in MZ twins vs. DZ twins confirms the importance of genetic factors in determining IQ. The weaker correlation between zygosity and autistic behaviors, reveals that epigenetic factors may play a role in determining these parameters. Alternatively, the ADI and ADOS may not be adequate quantitative test measures.

**Age of parents and the risk of cleft lip and palate.** *T. Ellerhorst, C. Owens, H. Oh, J. Mir, T. Mosby, M. Tolarova.*  
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Several epidemiological studies have suggested that the age of parents at the time of child's birth may play an important role in the etiology of congenital anomalies. Recent studies have associated a high parental age with sporadic retinoblastoma, prostate cancer, breast cancer, Down syndrome, and Alzheimer disease.

In order to explore the parental age as a possible risk factor for nonsyndromic cleft lip and/or palate (NSCL/P), we analyzed the age of parents in 123 children with NSCL/P (cases) and in 81 unaffected children (controls).

The median age of cases and controls was the same (13.9 vs. 14.1 years). However, we found a significant difference between the mean maternal age of cases and controls (25.8 vs. 26.4;  $P=0.005$ ), as well as between the mean paternal age of cases and controls (28.7 vs. 31.4;  $P=0.000003$ ). Mothers who were 20 years old and younger and also fathers who were 20 years old and younger had the highest risk of having a child affected with NSCL/P. Detailed analysis of epidemiological characteristics was done for subgroups according to cleft diagnosis. A strong relationship was found between the young paternal age and unilateral NSCL/P ( $P=0.006$ ). A similar association was found between the young age of mothers and unilateral NSCL/P ( $P=0.014$ ) and bilateral NSCL/P ( $P=0.022$ ).

The data from two different South American sites in Chile (Antofagasta and Chillan) were then compared. In both samples, one half of the mothers who gave birth to a child with bilateral NSCL/P were 24 years old or younger. Similar studies are currently being conducted in other areas of South America. The results of such studies will help to understand etiology of cleft anomalies in South America and thus enable us to develop preventive measures to decrease the prevalence of these serious congenital anomalies.

The field work for this study was supported by funding from ROTAPLAST International, Inc.



Program Nr: 1211 from the 2001 ASHG Annual Meeting

**Power of Cellular Automata for Identifying Gene-Gene and Gene-Environment Interactions.** *J.H. Moore, L.W. Hahn.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

We have previously developed a cellular automata (CA) approach to identifying gene-gene and gene-environment interactions (Moore and Hahn, in press). Briefly, CA are dynamic systems that consist of arrays of discrete cells. The state of a cell at each time step is determined by the current states of neighboring cells. CA can be used to perform computations by taking advantage of features such as massive parallelism. We have adapted a CA to accept an array of genotypes and/or environmental classes as input and produce an array as output that can be used to classify sibs as affected or unaffected (e.g.  $f(010110|\text{affected}) = 111111$  or  $f(100010|\text{unaffected}) = 000000$ ). CA models are optimized using machine learning and evaluated using 10-fold cross-validation and permutation testing. The goal of this study was to evaluate the power of CA models in the presence of noise. Using four different epistasis models, we simulated discordant sib-pairs with 5% genotyping error, 5% phenocopy, 20% phenocopy, or 50% genetic heterogeneity. We find that the CA approach retains > 95% power in the presence of genotyping error or phenocopies and > 75% power in the presence of genetic heterogeneity. We conclude that the CA approach is robust to common sources of noise and should be added to the repertoire of methods for identifying and characterizing gene-gene and gene-environment interactions.

**Statistical Analysis of Genotypic Association Data with HAPMAXII.** *M.L. Hamshere<sup>1</sup>, P.J. Giles<sup>1</sup>, M. Krawczak<sup>2</sup>, M.J. Owen<sup>1</sup>.* 1) Psychological Medicine, UWCM, Cardiff, UK; 2) Medical Genetics, UWCM, Cardiff, UK.

HAPMAXII is a software package that facilitates the analysis of genotypic association data in population- and family-based samples (<http://psychmed.uwcm.ac.uk/hapmaxii>). HAPMAXII is an extension of the original HAPMAX (Krawczak et al., *Hum Genet* 1988). The software is designed so that both trio and case-control data sets can be included in the same analysis. Markers on autosomal and sex chromosomes, including deleted regions can be incorporated into the analysis. An EM algorithm (Dempster et al., *J. R. Stat. Soc. B* 1977) is employed to obtain maximum likelihood estimates of the haplotype frequencies. A choice of start vectors is available to include the phase-unknown individuals into the analysis. The user can then investigate the sensitivity of the haplotype frequency estimates to the start vector. Likelihood ratio statistics and analytical and empirical significance levels are produced. A range of other statistics can be supplied by HAPMAXII, including the Lewontin's disequilibrium coefficient  $D'$  (Hedrick, *Genet* 1987) and a test for Hardy Weinberg equilibrium in population-based data (Guo and Thompson, *Biometrics* 1992).

**Genetic background cluster analysis : theory and applications.** *B.A. Thiel<sup>1</sup>, S. Kardia<sup>4</sup>, M. Province<sup>5</sup>, D.C. Rao<sup>5</sup>, N.J. Schork<sup>1,2,3</sup>.* 1) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Program for Population Genetics and Department of Biostatistics, Harvard University School of Public Health, Boston, MA; 3) The Jackson Laboratory, Bar Harbor, ME; 4) Department of Epidemiology, University of Michigan, Ann Arbor, MI; 5) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO.

Population samples (families, sibpairs, individuals, etc.) ascertained for gene mapping studies are often assumed to be largely independent, representative, and homogenous with respect to the population from which they are drawn. Testing these assumptions can be difficult without any *a priori* knowledge of possible sources of non-independence or genetic background heterogeneity. We describe a suite of strategies for testing the genetic heterogeneity of a sample of individuals. The proposed techniques make use of genotype data collected on sampled individuals at markers distributed throughout the genome. The strategies are flexible and can be used for a variety of purposes including controlling for cryptic stratification in genetic association studies, reconstructing genealogies and genetic relationships, and investigating human molecular diversity. Although we focus on important methodological detail, such as the determination of the number of clusters in a sample and analytic techniques used to determine the clustering (such as factor analysis and other similarity matrix-based cluster methods), we also provide an example of these methods with large genetic epidemiological samples from the NHLBI Family Blood Pressure Program. We also discuss the shortcomings of our strategy as well as areas for further research.

Program Nr: 1214 from the 2001 ASHG Annual Meeting

**Machine Learning Optimization of Neural Network Architecture Improves the Identification of Gene-Gene and Gene-Environment Interactions.** *M.D. Ritchie, J.S. Parker, L.W. Hahn, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

Neural networks (NN) have been proposed as a method for detecting and characterizing gene-gene and gene-environment interactions. However, most applications of NN use a fixed set of genetic loci as input and a fixed number of hidden layer nodes and node connections. With these NN, only the weights of the network connections are optimized. This greatly limits the flexibility of the NN that in turn limits the power of the NN to identify functional loci. The goal of this study was to develop a NN approach that uses machine learning for optimizing both the selection of genetic loci as input and the NN architecture in addition to the NN weights. Essentially, this method evolves the structure of the neural network and identifies the functional loci out of a pool of many candidates. The best models identified by the NN are then evaluated using 10-fold cross validation and permutation testing. Using simulated data, we have demonstrated that this approach improves the identification of gene-gene interactions and facilitates interpretation of multilocus results. This study supports the idea that NN will be an important statistical tool for the study of complex multifactorial diseases.

**The power of whole genome association studies that exploit linkage disequilibrium.** *N.J. Schork*<sup>1,2,3</sup>. 1) Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Program for Population Genetics and Department of Biostatistics, Harvard University School of Public Health, Boston, MA; 3) The Jackson Laboratory, Bar Harbor, ME.

There is a great deal of controversy surrounding whole genome association studies for mapping disease-predisposing genes. Much of this controversy is rooted in an ignorance about the strength of linkage disequilibrium (LD) between alleles at neighboring polymorphic loci. LD strength can basically be used to gauge the probability that observed marker locus alleles will act a good "surrogates" for unobserved disease relevant alleles. Both theoretical and empirical studies have been pursued to assess LD strength and the results of these studies vary considerably.

We present a method for assessing the power of association studies that uses actual data on LD strength. Our strategy uses novel theoretical constructs to account for uncertainty in LD strength (among other unknown parameters) and can be used to assess population-specific and genome-region specific association studies in addition to whole-genome studies. Our proposed methods are flexible and more compelling than traditional strategies for answering such questions as: "how dense a map of markers is needed for a genome-wide association study" or "what is the likely error rate associated with genetic association studies that exploit LD?" Numerous examples are given that make use of data in the public domain.

**Fifteen years after Chernobyl accident: the analysis of genetic consequences registered in the population of Belarus.** *G.I. Lazjuk<sup>1</sup>, P. Verger<sup>2</sup>, E. Robert<sup>3</sup>, H.G. Ilyina<sup>1</sup>, I.A. Kirillova<sup>1</sup>, Zh.P. Kravchuk<sup>1</sup>, I.V. Novikova<sup>1</sup>, I.V. Naumchik<sup>1</sup>, N.V. Rumyantseva<sup>1</sup>.* 1) Belarus Institute for Hereditary Diseases, Minsk, Belarus; 2) Institute de Protection et de Surete Nucleaire, Paris, France; 3) European Institute of Genomutations, Lyon, France.

Since 1982 Belarus National Registry (BNR) for obligatory registered congenital malformations (ORCM) monitors annually about 90,000 births and over 2000 pathologically examined medical abortuses (MA). The Registry recorded a significant increase of ORCM and embryonic anomalies found in MA, in the population of Belarus (Lazjuk G. et al., 1993, 1995, 1996). The most significant increase was registered in the areas with Cs<sup>137</sup> contamination density over 555kBq/km<sup>2</sup>.

To determine the factors causing this increase, the studies have been started within International Health Effect project, with Belarus Institute for Hereditary Diseases (Minsk, Belarus), Institute de Protection et de Surete Nucleaire (Paris, France), and European Institute of Genomutations (Lyon, France) as participating sides.

The main preliminary conclusions:

1. The data stored at BNR can be used for the analysis of CM dynamics, since the methods of data collection and CM nomenclature suit EUROCAT recommendations, and the figures reflecting the completeness of ORCM in the contaminated areas before and after the accident differ only slightly.

2. In Belarus a number of pregnancies with abnormal embryonic development is being increased.

3. Recently the differences between the rates of increase of ORCM frequencies in the control and contaminated areas were becoming smaller, however, the total ORCM frequency for 13-year period was recorded to be 12% higher in the contaminated areas than in the control ones.

**Meckel syndrome in Northern Ireland - evidence for possible segregation distortion?** *A.C. Magee, N.C. Nevin.*  
Regional Genetics Service, Belfast City Hospital Trust, Belfast, Northern Ireland.

Meckel syndrome (MS) - first described in 1822 - is one of the well-recognised, autosomal recessive, multiple congenital anomaly syndromes. The main diagnostic criteria comprise occipital encephalocele, polydactyly, cystic changes in the kidneys and hepatic fibrosis. The ascertainment and epidemiology of MS in Northern Ireland (NI) suggest the possibility of segregation distortion, which has been described in other Mendelian inherited conditions. From 1977 to 2000, 27 cases of MS (11 male, 13 female, 3 sex not determined) have been retrospectively ascertained in 10 families. The diagnosis was confirmed by post-mortem in at least one case from each family. The incidence of MS in NI is therefore estimated at 1/25,000. All couples, except one of Indian origin, were Northern Irish. In 2 families, the parents were consanguineous, being first cousins. Pedigrees were obtained for all families. It was noticeable that these parents seemed to have a higher than expected recurrence of MS. Pregnancy rate in the families ranged from 3 to 10. The total number of pregnancies was 63, with 12 first trimester miscarriages. There were 24 healthy sibs (9 male, 15 female). Thus, 27 of a total of 51 sibs (53%) had MS. When this is corrected for truncate single ascertainment, by eliminating one case from each sibship, the proportion of affected sibs is 33.3%. This is significantly higher than the 25% expected with autosomal recessive inheritance and suggests segregation in favour of the Meckel allele.

**Methylenetetrahydrofolate Reductase and Congenital Heart Defects: Evidence for Gene - Environment**

**Interaction.** *J.S. Zeiger<sup>1</sup>, J.B. Hetmanski<sup>1</sup>, I. McIntosh<sup>1</sup>, C. Loffredo<sup>2</sup>, T.H. Beaty<sup>1</sup>.* 1) Johns Hopkins Medical Institutions, Baltimore, MD; 2) Georgetown University, Washington, DC.

**Purpose:** Congenital heart defects (CHD) represent a major public health issue; collectively they are the most common birth defect creating a considerable medical, emotional and economic burden. This case-control study tested for the possible involvement of two polymorphisms in the gene encoding for methylenetetrahydrofolate reductase (MTHFR), C677T and A1298C, in the etiology of congenital heart defects.

**Methods:** Four types of heart defects were examined: aortic stenosis (AS, n=33), coarctation of the aorta (CoA, n=56), pulmonic stenosis (PS, n=72), and atrial septal defects (ASD, n=62). Cases and controls (n=308) for this study were drawn from the Baltimore-Washington Infant Study, a population based study conducted between 1981 and 1989 to examine risk factors for CHD. Odds ratios with 95% confidence intervals were calculated to test for associations between genotype and case/control status, and to test for interactions between genotype and environmental factors. Separate analyses were performed for Caucasians and African-Americans due to the differences in their allele frequencies.

**Results:** There were no differences between cases and controls for either of the MTHFR variants. Heavy alcohol use (OR = 6.76, 95% CI = 2.61-17.51) and heavy smoking (OR = 4.70, 95% CI = 1.38-16.04) increased risk for PS among Caucasians. There was some evidence of interaction between smoking and the A1298C genotype among African American PS cases (OR nonsmokers = 0.98, 95% CI = 0.27-3.42; OR smokers = 3.03, 95% CI = 1.09-8.52).

**Conclusions:** Smoking may be a risk factor for congenital heart defects alone and in the presence of the MTHFR A1298C variant.



**Genetic disease in offspring of survivors of childhood and adolescent cancer.** *J.J. Mulvihill<sup>1</sup>, L.C. Strong<sup>2</sup>, L.L. Robison<sup>3</sup> and Investigators of the Childhood Cancer Survivor Study.* 1) Department of Pediatrics, Children's Hospital Oklahoma, Oklahoma City, OK/JNCI; 2) The University of Texas M.D. Anderson Cancer Center, Houston, TX; 3) Department of Pediatrics, University of Minnesota, Minneapolis, MN.

No environmental agent has been proved to cause human germ cell mutation seen as genetic disease in offspring. Cancer survivors often receive intensive chemotherapy and radiotherapy that cause human and experimental somatic mutations and animal germline mutations. To study environmental germline mutagenesis, we used the Childhood Cancer Survivor Study, a retrospective cohort of 14,054 children diagnosed with common cancers before age 21 years and surviving at least 5 years, at 25 US and Canadian institutions (JNCI 2001;93:618). Participants were 54% male, 87% white, and 64% between ages of 20 and 39 years at follow-up; 44% received combination surgery, radiotherapy, and chemotherapy. Genetic disease in patients, families, and offspring were ascertained by self-administered questionnaires; verification by records is underway. Genetic and congenital disease occurred in 158 (3.7%) of 4,214 offspring of survivors, compared with 102 (4.4%) of 2339 offspring of controls; there were no apparent differences in the proportion of offspring with cytogenetic syndromes, single-gene defects, or simple malformations. These preliminary results, if confirmed after verification, provide reassurance that cancer treatment using modern protocols does not carry a large risk for genetic disease in offspring conceived many years after treatment. (NIH grant CA55727).

**MUTATIONAL HOTSPOTS AND HYPERVARIABLE NUCLEOTIDE MOTIFS IN THE NON-CODING HUMAN mtDNA CONTROL REGION.** *B. Malyarchuk, M. Derenko.* Genetics Lab, Biological Probl of the North, Magadan, Russia.

It is not known why the hypervariability of certain mtDNA sites exists. It seems likely that DNA context predisposes to mutation processes, leading to point mutations, deletions and duplications. It is possible that a certain nucleotide motifs are responsible for increased variability of the human mitochondrial genome. To test this hypothesis, we have performed a search for short hypervariable motifs based on analysis of mtDNA control region variation data in human populations. In order to determine hypervariable sites among nucleotide positions in the noncoding region, we have analyzed the frequency of appearance of identical mutations in mtDNA sequences belonging to the different phylogenetic haplogroups of mtDNAs. As a result, 14 nucleotide positions, where mutations appeared independently in 6-11 of 15 mtDNA haplogroups analyzed, were found in the 360 base pair segment of the mtDNA non-coding region. The contextual analysis of the hypervariable positions distribution has allowed to reveal at least three types of nucleotide motifs, which are characterized by increased level of mutagenesis in respect to single-nucleotide substitutions - GTAC (for nps 16093, 16129, 16304, 16311), ACCC (for nps 16172, 16256, 16265, 16291, 16294, 16298, 16390) and CCTC (for nps 16189, 16356, 16362). It was found that these motifs are often associated with direct repeated sequences, including tandem repeats. Moreover, the hypervariable motifs CCTC and ACCC as components of the poly-C tracts are the part of direct repeats, leading both to deletions and tandem duplications in human mtDNA. The data received allow us to conclude that the human mitochondrial genome instability is caused considerably by mutation processes predetermined by DNA context. The similarity of the hotspot point mutations with regard to the position and kinds of mutations in different mtDNA haplogroups and the predominance of the transitional hotspots allow to suggest that these mutations are primarily spontaneous in origin and arise mostly from DNA replication error induced by certain mtDNA nucleotide motifs. This work was supported by grants of RFBR (00-06-80448) and Frontiers in Genetics (99-04-30).

**The Arab Genetic Disease Database: A National Database of Genetic Disorders.** A.S. Teebi<sup>1</sup>, S.A. Teebi<sup>2</sup>, C.J. Porter<sup>2</sup>, A.J. Cuticchia<sup>2</sup>. 1) Division of Clinical and Metabolic Genetics, Department of Pediatrics, Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Bioinformatics, Hospital For Sick Children, Toronto, ON, Canada.

The Arab Genetic Disease Database (AGDDB) is a curated catalogue of genetic disorders found in Arab populations. The Arabs are a highly diverse set characterized by high rates of consanguineous marriages, increased frequencies of autosomal recessive disorders and elevated frequencies of new disorders and variants. Until recently, they have been poorly studied. The textbook *Genetic Disorders among Arab Populations* (Teebi and Farag, 1997, OUP) was the first serious attempt to compile the existing data on the subject, and subsequently generated a large amount of interest and research on disorders of Arab populations. The first release of AGDDB is based on information in that book, which included data from disparate, often unrefereed/indexed sources. The AGDDB data schema is relational, comprised of key data elements and their important attributes. It is centered around the element of the Disorder Report, with elements for Clinical data, Genomic data (including protein), Population data and Reference data also present. Population-specific variants and detailed frequency reporting is included. The Arab Genetic Disease Consortium (30 investigators, 18 countries) is responsible for annotating, editing and reviewing AGDDB data. When the initial indexing of data is finished, AGDDB will contain over 1000 unique disorder entries. Entries are cross-linked with their counterparts in OMIM and GDB, and similar associations to relevant locus-specific and central mutation databases are in the works. The database is queriable by keyword across all its fields, and logical operators are allowed. More focused searches may be performed on disorder, gene name, OMIM number, population and citation. The first release of AGDDB will be freely accessible at <http://www.agddb.org/> in September of 2001. AGDDB is the first population-specific variation and disorder database, and can serve as a robust prototype for the cataloguing of such data in other populations.

**Y-CHROMOSOME POLYMORPHISMS AND THE PEOPLING OF GRAN CANARIA.** *D. Castro-de-Guerra*<sup>1</sup>, *M. Bortolini*<sup>2,3</sup>, *M. Thomas*<sup>2</sup>, *J. Aguilar*<sup>2</sup>, *L. Angel*<sup>1</sup>, *F. Sánchez-García*<sup>4</sup>, *J. Rodríguez-Gallego*<sup>4</sup>, *M. Déniz-Naranjo*<sup>4</sup>, *J. Soria*<sup>5</sup>, *J. Arellano*<sup>6</sup>, *A. Auóon*<sup>7</sup>, *F. Salzano*<sup>3</sup>, *N. Bradman*<sup>2</sup>, *A. Ruiz-Linares*<sup>2</sup>. 1) Laboratorio de Genética Humana, IVIC, Caracas, Caracas, Venezuela; 2) The Galton Laboratory, Department of Biology, University College London, UK; 3) Dep. de Genet., UFRGS; 4) Servicio de Inmunol., Hosp. de Gran Canaria, Gran Canaria; 5) Dep. d Hematol., Hosp. de la Santa Creu i Sant Pau, Spain; 6) Centro de Cien. de la Salud, Trasera Hosp. Insular, Gran Canaria; 7) Inst. de Med. Trop., Spain.

The Canary Islands were initially colonized around 4,000 years ago by people probably originating in North West Africa. In the past few centuries, Europeans and Sub-Saharan Africans have moved into the islands as a consequence of the Spanish colonial expansion. To evaluate the origin of Canarian male lineages and examine the history of admixture of this population we typed four Y-biallelic markers: DYS271, DYS287, M9, 92R7, and six Y-STRs: DYS19, DYS388, DYS390, DYS391, DYS392, DYS393 in 196 samples from Gran Canaria, Spain and Morocco. We observed that the majority (83%) of Canarian Y-chromosomes are of Spanish origin, but an important North African contribution was also detected (17%). Contrasting mtDNA and Y data sets corroborates a history of directional mating involving preferentially Spanish males and native Canarian females. A similar trend has been reported for other areas of the former Spanish Empire although considerable regional variation is likely to exist and is illustrated by comparing our findings in Gran Canaria with those from Antioquia (Colombia).

**Contribution of UCSNP-43 and UCSNP-19 within the Calpain-10 gene to risk of type 2 diabetes stratified by environment.** *J.M. Norris<sup>1</sup>, C.D. Engelman<sup>1</sup>, M.M. Barmada<sup>2</sup>, R.E. Ferrell<sup>2</sup>, L.K. Moss<sup>1</sup>*. 1) Dept. of Preventive Medicine and Biometrics, Univ of Colorado Health Sciences Center, Denver, CO; 2) Dept. of Human Genetics, Univ of Pittsburgh Graduate School of Public Health, Pittsburgh, PA.

Type 2 diabetes may result from an interaction between genetic susceptibility and environment. The single-nucleotide polymorphisms, UCSNP-43 and UCSNP-19, located in the calpain-10 gene on chromosome 2 (calpains are a family of non-lysosomal cysteine proteases) have shown linkage and association with diabetes in an Hispanic-American population. In addition, epidemiological studies have found an association between diabetes risk and both high dietary fat and low physical activity. We used the transmission/disequilibrium test (TDT) and the sib TDT (s-TDT) to look for linkage to type 2 diabetes in 859 Hispanic individuals from families living in the San Luis Valley in Colorado. This analysis performs a TDT when parental data are available, and a s-TDT when they are not, and calculates a combined z-score ( $z'$ ). We also stratified the analyses by dietary fat and physical activity levels of the diabetic proband to examine potential gene-environment interactions. UCSNP-43 and UCSNP-19 were measured in the laboratory of Dr. R.E. Ferrell. Dietary intake over the past year was collected from the diabetic proband using the Block food frequency questionnaire. Lifetime (prior to diabetes diagnosis) occupational and leisure physical activity was collected using the Kriska Physical Activity Questionnaire. Overall, the TDT/s-TDT found no linkage of either UCSNP-43 or UCSNP-19 to type 2 diabetes in these Hispanic-American families ( $z' = 0.629$  and  $1.089$ , respectively). Stratification by dietary fat and physical activity levels resulted in similar non-significant results. In summary, UCSNP-43 and UCSNP-19 are not linked to diabetes in our population, nor does there appear to be a gene-environment interaction between these SNPs and diet or physical activity in the etiology of type 2 diabetes.

**Genotype and phenotype correlations in oligozoospermic and azoospermic men with Y chromosome**

**microdeletions.** *K. Kucheria*<sup>1</sup>, *K. McElreavey*<sup>2</sup>, *R. Dada*<sup>1</sup>. 1) Dept Anatomy, Div Genetics, All India Inst Medical Sci, New Delhi, India; 2) Institut Pasteur, Immunogenetique Humaine, Paris, France.

10-15 % of couples encounter difficulty to procreate. In 40-50% of these cases the male partner has qualitative or quantitative abnormalities of sperm production. Spermatogenic failure is associated with microdeletion of the long arm of the Y chromosome. Three regions AZFa, AZFb and AZFc on long arm of Y chromosome are critical for spermatogenesis. These 3 loci act at different stages of germ cell development and deletion of each results in a characteristic phenotype. Deletion of AZFa, AZFb, AZFc regions results in Sertoli Cell Only syndrome (SCO), maturation arrest and hypospermatogenesis respectively. One hundred and two infertile males with oligozoospermia and azoospermia were analysed for cytogenetic and molecular profile. Cytogenetic and semen analysis was done in each case. Testicular Fine Needle aspiration Cytology was collected whenever possible. In 70 cytogenetically normal cases, microdeletion analysis was done using STS-PCR approach using primers sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). The STS was considered as absent after 3 amplification failures. Eight of the 70 cases had deletion of at least one of the AZF locus. Four cases had AZFc deletions, three cases had AZFa and AZFb deletions and one case showed AZFb deletion alone. Two cases with AZFa+AZFb deletions had SCO syndrome and 1 case of AZFc deletion showed hypospermatogenesis. In the present study the frequency of microdeletion was 9.9 %. This figure is similar to that reported in three different European populations (Italian, French and Danish) suggesting that, the worldwide incidence of Y microdeletions is likely to be similar, if a common clinical criteria and marker set are used. Thus in a significant number of idiopathic cases of male infertility there is an underlying genetic cause. Detection of these microdeletions aids in better management of these infertile couples should they opt for Assisted Reproductive Technology.

**Genetics of male infertility in Iran.** *C. Krausz<sup>1</sup>, L. Quintana-Murci<sup>1</sup>, H. Sayar<sup>2</sup>, K. McElreavey<sup>1</sup>*. 1) Immunogenetique Humaine, Institut Pasteur, Paris, France; 2) Blood Transfusion Service, Tehran, Iran.

An analysis of the Y chromosome was performed on DNA samples from 46 men with idiopathic male infertility (azoospermia and severe oligozoospermia) of Iranian origin. Microdeletions of the Y chromosome were found in 11 % of Iranians. All microdeletions were in the AZFc region. Y chromosome microdeletions were not observed in a control group of more than 500 individuals from this region. These results are consistent with the microdeletion frequencies that we have previously reported in European populations (France, Italy and Denmark), where a common clinical and molecular protocol was followed. In the Iranian population, consanguinity was noted in 26% of all cases, including one case with a Y microdeletion. A family history of infertility was observed in 17% of Iranian cases including one large family with a case of premature ovarian failure. Inter- and intrafamilial variability of the infertile phenotype was observed. The transmission of the phenotype suggests genetic heterogeneity, and is compatible with X-linked, autosomal dominant and autosomal recessive sex-limited modes of inheritance. The data suggest that familial cases of infertility may be more common than previously supposed.

**Identification of genetic risk factors associated with infertility. A Y chromosome haplogroup is associated with reduced sperm counts.** *K. McElreavey<sup>1</sup>, L. Quintana-Murci<sup>1</sup>, E. Rajpert-De Meyts<sup>2</sup>, N. Jørgensen<sup>2</sup>, M. Jobling<sup>3</sup>, Z. Rosser<sup>3</sup>, N. Skakkebaek<sup>2</sup>, C. Krausz<sup>1</sup>.* 1) Immunogenetique Humaine, Inst Pasteur, Paris, France; 2) University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark; 3) Department of Genetics, University of Leicester, Leicester, U.K.

In man, infertility is associated with microdeletions of specific regions of the long arm of the Y chromosome. This indicates that factors encoded by the Y chromosome are necessary for spermatogenesis. However; the majority of men with either idiopathic azoospermia or oligozoospermia have a grossly intact Y chromosome and the underlying cause of their infertility is unknown. We hypothesised that some of these individuals may carry other rearrangements or sequence variants on the non-recombining region of the Y chromosome that may be associated with reduced spermatogenesis. To test this hypothesis, we typed the Y chromosome in a group of Danish men with known sperm counts and compared the haplotype distribution with that of a group of unselected Danish males. We found that one class of Y chromosome, referred to as haplogroup 26+, was significantly overrepresented (27.9%;  $P < 0.001$ ) in the group of men with either idiopathic oligozoospermia (defined as  $< 20 \times 10^6$  sperm/ml) or azoospermia compared to the control Danish male population (4.6%). This study defines, for the first time, a class of Y chromosome that is at risk for infertility in a European population. This observation suggests that selection may be indeed active on the Y chromosome, at least in the Danish population. We are currently extending this study to include other populations in Germany, Iran, Turkey and India. The relationship between the Y chromosome background and various semen parameters, including high sperm counts, is also being tested in Danish and Finnish populations. This observation suggests that selection may be indeed active on the Y chromosome, at least in the Danish population, raising the possibility that it could alter the pattern of Y chromosome haplotype distribution in the general population.



**Estimation of individual admixture in Trinidad: application to a case-control study of systemic lupus**

**erythematosus(SLE).** *M. Molokhia*<sup>1</sup>, *A.L. Patrick*<sup>2</sup>, *E. Parra*<sup>3</sup>, *J. Ye*<sup>3</sup>, *M.D. Shriver*<sup>3</sup>, *A.J. Silman*<sup>4</sup>, *P.M. McKeigue*<sup>1</sup>. 1) Epidemiology Unit, London School of Hygiene & Tropical Medicine, London,UK; 2) Ministry of Health, Port of Spain, Trinidad & Tobago; 3) Anthropology Department, Penn State University, State College, Pennsylvania, US; 4) ARC Clinical Sciences Unit, University of Manchester, Manchester,UK.

Objective: To distinguish between genetic and environmental explanations for high risk of SLE in west Africans compared with Europeans by studying the relation of risk to individual admixture. Methods: Cases of SLE, together with controls matched for age, sex and neighbourhood, were sampled from northern Trinidad, excluding those with Indian or Chinese names. Individuals were typed with a panel of 30 SNP markers chosen to have large frequency differentials between the three non-Asian parental populations (west African, European, Native American). Individual admixture (proportion of the genome that has ancestry from each parental population) was estimated from the marker genotypes. Results: This analysis was restricted to the 44 cases and 77 controls who reported that they had no Indian or Chinese ancestry and had been genotyped. Mean proportion of African admixture (M) was estimated in a Bayesian analysis using Markov chain simulation as 0.78 in cases, 0.71 in controls ( $p=0.04$ ). In a logistic regression analysis, the risk ratio associated with unit change in M (from 0 to 1) was estimated as 15.8 (95% CI 1.1 - 227). This association was strengthened after adjustment in the analysis for socioeconomic factors and for religious affiliation. Conclusion: Risk of SLE in this admixed population is strongly related to the proportion of west African admixture, consistent with a genetic explanation for the higher risk of SLE in west Africans compared with other ethnic groups. This result provides a basis for designing studies to localize the genes underlying this effect by studying people of mixed descent using a panel of markers across the entire genome.

**Complex segregation analysis of obsessive-compulsive disorder in families with pediatric probands.** *G.L. Hanna<sup>1</sup>, T.E. Fingerlin<sup>2</sup>, J.A. Himle<sup>1</sup>, G.C. Curtis<sup>1</sup>, K.R. Chadha<sup>1</sup>, D.Q. Koram<sup>1</sup>, M. Boehnke<sup>2</sup>.* 1) Dept Psychiatry, Univ Michigan, Ann Arbor, MI; 2) Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

Obsessive-compulsive disorder (OCD) is a psychiatric disorder affecting one percent to three percent of the population. Evidence from twin and family studies supports a genetic etiology for OCD. Three previous segregation analyses have implicated a gene of major effect in OCD. The purpose of this study was to establish a more precise mode of inheritance for OCD in families ascertained through pediatric probands. Complex segregation analyses of 52 families (35 case and 17 control) were performed by using regressive models as programmed in REGTL in the S.A.G.E. package. A total of 654 individuals were used in the analyses. Both definite and subthreshold OCD were considered affected. In analyses without gender effects, the environmental and sporadic models were clearly rejected. The dominant and additive models were also rejected, whereas the general Mendelian (codominant) and recessive models were consistent with the observed data. In analyses with gender effects, the environmental and sporadic models again were strongly rejected, as were the dominant, additive, and recessive models. The general Mendelian (codominant) model remained the most parsimonious explanation for the observed data. The results provide further evidence for a major locus in the etiology of OCD.

**The effect of parental age on the occurrence of Neurofibromatosis 1.** *J. Tzenova*<sup>1</sup>, *H. Joe*<sup>2</sup>, *V.M. Riccardi*<sup>3</sup>, *J.M. Friedman*<sup>1</sup>. 1) Department of Medical Genetics; 2) Department of Statistics, University of British Columbia, Vancouver, BC, Canada; 3) The Neurofibromatosis Institute, La Crescenta, CA.

New mutations account for half of all patients with neurofibromatosis 1 (NF1), and about 80% of new mutations occur in the paternally-inherited allele. The exception is large deletions, which are predominantly maternal in origin. Typical NF1 may also occasionally result from somatic mutations. Previous studies of paternal age among patients with sporadic NF1 have been inconclusive. We postulated that failure to find a paternal age effect in these studies may have resulted from inclusion of patients with large deletions and somatic mutations, for whom no paternal age effect would be expected.

In order to test this possibility, we used data collected from 280 sporadic and 389 familial NF1 patients. We excluded 11 (3.9%) exceptionally mild sporadic cases as possible somatic mosaics and 14 (5.0%) sporadic cases with the large deletion phenotype. We compared paternal age in the remaining 255 sporadic probands to 100 familial probands for whom both the paternal and maternal ages at birth were known. The mean paternal age for the sporadic probands (31.71 years) was significantly greater ( $p = 0.017$ ) than that for familial probands (29.87 years). The results were unaffected by ethnicity or year of ascertainment.

We also studied the effect of maternal age on transmission of the abnormal *NF1* allele. We found no significant difference between the maternal age at birth of 74 proband children of affected mothers and that of 63 proband children of affected fathers. Logistic regression of affection status in 334 children of affected women and 146 children of affected men in 239 families showed no significant relationship with gender of the affected parent, maternal age at birth of the child, paternal age at birth of the child, or birth order. Probands were excluded from this analysis to reduce ascertainment bias. We conclude that there is suggestive evidence for a paternal age effect on the occurrence of sporadic NF1 but that neither maternal nor paternal age at birth affects the occurrence of familial NF1.

**Mutation analysis of the *BRCA1*-interacting genes *ZBRK1* and *BRIP1* among *BRCA1/2*-negative probands from breast/ovarian cancer families.** *J.P. Struewing, J.L. Rutter, M.R. Dávila, A.M. Smith, M.H. Greene, M.A. Tucker.* Div Cancer Epidemiology & Genetics, NCI, NIH, Bethesda, MD.

Two potential breast cancer susceptibility genes, encoding the *BRCA1*-interacting proteins *ZBRK1* and *BRIP1* (originally termed *BACH1*) have recently been identified in yeast two-hybrid screens using *BRCA1* as bait. Previous mutational analysis of *ZBRK1* has not been reported, but analysis of *BRIP1* among 65 early-onset breast cancer cases identified two missense mutations. By aligning the Genbank mRNA records of *ZBRK1*, encoding a zinc finger containing protein and *BRIP1*, encoding a helicase, with genomic fragments, we developed PCR primers to amplify the 4 exons of *ZBRK1* and the 20 exons of *BRIP1* from genomic DNA. We sequenced the resulting PCR amplicons from probands from 22 families with potentially inherited breast/ovarian cancer, all of whom were negative for *BRCA1/2* mutations. Families had at least one case of male breast cancer, two cases of ovarian cancer, or three or more cases of breast and ovarian cancer. A *ZBRK1* missense mutation Gln393His was identified in two subjects, altering a conserved Gln residue in the 7<sup>th</sup> of eight C2H2 zinc finger motifs. It is not yet known whether this alteration segregates with cancer in these families. We also identified three silent *ZBRK1* mutations Asp35Asp, Cys236Cys, and Pro373Pro and a 3'UTR variant 2067C>T in 4 or more subjects each. For *BRIP1*, in addition to the common missense mutation P919S and the silent mutations E879E and Y1137Y previously described, we identified two intron variants in 11 subjects each. We also identified intron variants IVS5-31G>C in two probands, IVS14+26delT and IVS19+82T>A in one proband each, and a Gln540Leu missense mutation in a single proband. Gln540Leu is a non-conservative amino acid change identified in an individual with inflammatory breast cancer but was not identified in her three relatives with breast cancer who were tested for this variant. Based on sequencing of *ZBRK1* and *BRIP1* in 22 *BRCA1/2*-negative probands from inherited breast/ovarian cancer families, it appears unlikely that mutations in these genes account for a large fraction of inherited forms of breast cancer.

**Pancreatitis-associated human cationic trypsinogen gene (PRSS1) mutations as a vehicle for better understanding trypsinogen evolution and physiology.** *J.M. Chen, T. Montier, C. Ferec.* INSERM-EMI 01 15, Etablissement Français du Sang-Bretagne, Université de Bretagne Occidentale, and Centre Hospitalier Universitaire, 46 rue Félix Le Dantec, 29275 Brest, France.

Historically, trypsinogens are one of the most extensively studied models for protein structure and function and for evolution of multigene families. The identification of mutations in the human cationic trypsinogen gene (PRSS1) as a cause of hereditary pancreatitis (HP) has attracted renewed attention to this familiar enzyme. While this finding offers better means for diagnosis, family testing and treatment of the disease, we show how pancreatitis-associated PRSS1 mutations (eg., D22G, K23R, N29I, and R122H) can be used as a vehicle for better understanding trypsinogen evolution and normal physiology.

Based upon a critical evaluation of the current mutational and functional analysis data in the context of a comprehensive amino acid sequence comparison of trypsinogens from various vertebrate species, insights into the molecular evolution of trypsinogens can be seen in three stages. Firstly, activation peptide sequences are subject to strong selection pressure to minimize trypsinogen autoactivation in higher vertebrates. Secondly, the R122 autolysis site, which is supposed to act as a "fail-safe" mechanism against intrapancreatic zymogen activation, has evolved in mammals. Thirdly, evolutionary divergence from T to N at residue 29 provides additional advantage in the human cationic trypsinogen. These observations, when considered in conjunction with the current knowledge of the complicated biogenesis and biochemistry of trypsin(ogen), enabled us to propose that, in the human cationic trypsinogen, the strongly selected activation peptide as the first-line and the R122 autolysis site as the second-line of the built-in defensive mechanisms that the body has evolved against premature digestive enzyme activation within the pancreas, respectively, and the positively selected N29 in PRSS1 as an "amplifier" to the R122 "fail-safe" mechanism. Continued research of the PRSS1 gene in the disease may reveal further fascinating details of trypsinogen evolution and physiology.

**Characterization of b-globin haplotypes among an admixed, population-based cohort of newborns with sickle cell disease.** *D.C. Crawford*<sup>1</sup>, *M. Caggana*<sup>2</sup>, *C. Tempelis*<sup>3</sup>, *K.B. Harris*<sup>2</sup>, *F. Lorey*<sup>3</sup>, *C. Nash*<sup>4</sup>, *K. Pass*<sup>2</sup>, *R. Olney*<sup>1</sup>. 1) National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention; 2) Wadsworth Center, New York State Department of Health; 3) Genetic Disease Branch, California Department of Health Services; 4) Division of Health Assessment and Screening, Illinois Department of Public Health.

Sickle cell disease (SCD) is caused by a single mutation in the b-globin gene on chromosome 11. Current evidence based on b-globin haplotypes suggests that the HbS mutation arose only in Africa, more than once. Previous studies suggest that migration and subsequent admixture are responsible for some of the b-globin haplotype diversity sampled in North America. To better characterize this diversity in the United States, we identified 617 newborns in California, New York, and Illinois identified with SCD through newborn screening in 1992-1993. Using stored bloodspots, we genotyped 432 samples for the restriction site polymorphisms (RSPs) *XmnI*, *HindIII*-Gg, *HincII*-yb, *HincII*-d, and *HinfI*. Among African-American (n=744) and Hispanic chromosomes (n=64), 15 and six haplotypes were identified, respectively. All six haplotypes found in the Hispanic population were also found among African-Americans, suggesting that the origin of the HbS mutation found among these Hispanics is of African descent. For both populations, ~89% of chromosomes were represented by the same three haplotypes. Furthermore, the expected haplotype heterozygosity was similar between the two populations. For the African-American population, however, observed haplotype heterozygosity (0.493) was significantly lower than expected ( $p < 0.02$ ). Examination of the individual RSPs revealed that Hispanics had a greater expected heterozygosity at *HindIII*-Gg ( $0.488 \pm 0.06$ ) and *HincII*-d ( $0.417 \pm 0.06$ ) compared with African-Americans ( $0.340 \pm 0.02$  and  $0.279 \pm 0.02$ , respectively). Also, the African-American population had a statistically significant increase in observed heterozygosity at *XmnI* ( $p < 0.01$ ). These results probably reflect the recent, complex migration and admixture experienced by both populations in the United States rather than selective forces related to the HbS mutation.

**Mitochondrial DNA diversity among Altaians.** *M. Derenko*<sup>1</sup>, *T. Grzybowski*<sup>2</sup>, *B. Malyarchuk*<sup>1</sup>, *G. Denisova*<sup>1</sup>, *J. Czarny*<sup>2</sup>, *V. Kakpakov*<sup>3</sup>, *D. Miscicka-Sliwka*<sup>2</sup>, *I. Zakharov*<sup>3</sup>. 1) Genetics Lab, Inst Biological Prob of North, Magadan, Russia; 2) Institute of Forensic Medicine Ludwik Rydygier's University School of Medical Sciences, Bydgoszcz, Poland; 3) Vavilov Institute of General Genetics, Moscow, Russia.

To investigate the origin and evolution of aboriginal populations of Altai and Sayan region we present here mtDNA analysis (HVS-1 and HVS-2 sequencing combined with RFLP) of Altaians (n = 110) and compare them with the populations of North, East, Central Asia and West Eurasia that have historically had a great influence to Altaians. The Altaians studied exhibit the high percentage (48.2%) of haplogroup M (M\*, C, D, E, G, Z) mtDNA lineages. The overall pattern of the frequency distribution of the M mtDNA haplogroups is similar to the other East Asian populations where M haplogroup and its subhaplogroups comprise about 50% of mtDNA lineages. East Asian mtDNA haplogroups B and F were found in Altaians with the frequencies of 3.6% and 8.2%, respectively. Lineages, characteristic to western Eurasian mtDNA haplogroups (H, U, J, T, X, I) were found in Altaians at frequency 32.7%, with the haplogroup U lineages being prevalent (18%) and most substructured (U1, U2e, U3, U4, U5). The remaining 7.3% of Altaian mtDNAs comprise of the haplogroup N\* and N1a. Thus, the Altaians studied exhibited both Asian-specific (65%) and West Eurasian (35%) mtDNAs. Phylogeographic analysis shows that the Asian-specific component of Altaian mtDNA gene pool represented mostly by typically Siberian/Central Asian lineages whereas West Eurasian component has a more complicated structure. A considerable amount of these lineages have West Asian ancestry reflecting the imprints of the early Europeoid migrations along the Steppe Belt. Some of Altaian mtDNAs (from U2e, U4, and X groups) may have autochthonous origin testifying thereby the complexity of population history of Altai region. This work supported by grants from Russian Foundation for Basic Research (99-06-80430) and from the Ludwik Rydygier Medical University in Bydgoszcz (BW 90/01).

**Mitochondrial DNA polymorphism in the populations of Siberia and Central Asia: lineages of East-Eurasian and West-Eurasian origin.** *M.V. Golubenko<sup>1</sup>, T. Kivisild<sup>2</sup>, V.B. Salukov<sup>1</sup>, K.V. Puzyrev<sup>3</sup>, J.O. Soltobaeva<sup>4</sup>, V.N. Tadinova<sup>1</sup>, E.R. Eremina<sup>5</sup>, T.P. Muraviova<sup>1</sup>, V.P. Puzyrev<sup>1</sup>, R. Villems<sup>2</sup>.* 1) Institute of Medical Genetics, Tomsk, Russia; 2) Estonian Biocentre, Tartu, Estonia; 3) Institute of Cardiology, Tomsk, Russia; 4) Kyrghiz Medical Academy, Bishkek, Kyrghizstan; 5) Buriat div. of the Institute of paediatrics and Human Reproduction, Ulan-Ude, Russia.

MtDNA polymorphism has been investigated in 9 aboriginal peoples of Siberia and Central Asia (Tuvinians, Buriats, Northern Altai, Southern Altai, Yakuts, Kyrghis, Uzbeks, Tadjiks), by the screening of the restriction sites which determine the main mtDNA haplogroups (A, B, F, H, U, M: C, D); for the Altai, Uzbeks, Tadjiks, Kyrghiz, HVS-I sequences also have been determined. Total sample size was about 1200 individuals. In all populations the haplogroups of both West-Eurasian and East Eurasian origin are presented, but their frequencies vary broadly. The highest haplogroup diversity is observed in some Central Asian populations (Kirghiz, Uzbeks, Tadjiks). The populations, except Tadjiks, may be divided into two groups. In the populations of Kirghiz, Uzbeks, and Altai, two main branches of mtDNA tree are presented with considerable frequencies: the haplogroups of West-Eurasian origin (H, I, J, T, U, X) and those of East-Eurasian origin (A, B, F, M, N9). Siberian populations possess mainly lineages of Asian origin with prevalence of haplogroup M (70-80%). The frequency of West-Eurasian haplogroup H in Siberia is no more than 5%, as well as the frequency of haplogroup U (up to 8% in some local populations). The populations of Tuvinians, Buriats, Yakuts, Kyrghiz, Tadjiks, and Altai, show the presence of four founding Native American haplogroups (A, B, C, D). In Tadjiks, the total frequency of the lineages of East-Asian origin is about 10%. So the spreading of mtDNA lineages in North-East Eurasia is in accordance with anthropological affinity of the populations and with their territorial locations, with every ethnicity having specific pattern of haplogroups structure and frequencies.



**Y-associated Polymorphism in the Iranian Population.** *H. Najmabadi*<sup>1</sup>, *M.F. Hammer*<sup>2</sup>, *B. Moghimi*<sup>3</sup>, *A.R. Noorian*<sup>3</sup>, *M.R. Keikhaee*<sup>4</sup>, *P. Pasalar*<sup>5</sup>. 1) Dept Molecular Biol, Torrance, CA; 2) Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ; 3) Tehran University of Medical Sciences, School of Medicine, Tehran, Iran; 4) University of Welfare and Rehabilitation, Tehran, Iran; 5) Department of Biochemistry, Tehran University of Medical Sciences, Tehran, Iran.

There is a non-recombining region on the human Y chromosome which can be used to investigate human evolution during the last 200`000 years. This polymorphic region, which is highly conserved, includes the Y Alu insertional Polymorphism or YAP element (DYS 287), the poly (A) tail associated with the YAP element and a tetranucleotide microsatellite (DYS 19). In this study, we have collected DNA samples from seven major ethnic groups in Iranian population and determined its Y-associated polymorphic variation. Having analyzed the genetic diversity data, we can determine different patterns of evolutionary migrations in Iran.

**Y chromosome markers indicate a lack of clustering in the Hindu caste system.** *S.R. Indugula<sup>1</sup>, S. Mastana<sup>2</sup>, B. Su<sup>1</sup>, G. Sun<sup>1</sup>, S.S. Papiha<sup>3</sup>, B.M. Reddy<sup>4</sup>, P. Underhill<sup>5</sup>, R. Chakraborty<sup>6</sup>, L. Jin<sup>6</sup>, R. Deka<sup>1</sup>.* 1) Dept Environmental Hlth, Univ Cincinnati, Cincinnati, OH; 2) Loughborough University, Loughborough, England; 3) University of Newcastle upon Tyne, Newcastle, England; 4) Indian Statistical Institute, Calcutta, India; 5) Stanford University, Stanford, CA; 6) University of Texas Health Science Center, Houston.

The Hindu caste system is amongst the most complex social structures and its origin has been studied from various perspectives. Hierarchical stratification among the castes varies regionally, which is compounded by regional endogamy and linguistic diversity. We have studied 668 males from 16 regional populations from the Indian subcontinent, belonging to various caste groups, at 12 binary polymorphisms on the Y chromosome. Two haplogroups defined by markers M52C and M45A are predominant in all of the studied populations. Other haplotypes are shared across geographical boundaries and hierarchically defined varnas (caste status). A principal component analysis indicates a lack of clustering of individual varnas both within and between regions. Our initial observations are suggestive of past population movements within the subcontinent before the formation of the Hindu caste system together with population migrations from various routes contributing to the current complex population structure.

**Inter- and Intra-Island Genetic Diversity in Adriatic Populations of Croatia: Implications for Studying Complex Diseases in Isolated Populations.** *I. Martinovic Klaric<sup>1</sup>, L. Jin<sup>2</sup>, R. Chakraborty<sup>2</sup>, R. Deka<sup>2</sup>, L. Barac<sup>1</sup>, M. Pericic<sup>1</sup>, N. Smolej Narancic<sup>1</sup>, I. Rudan<sup>3</sup>, B. Janicijevic<sup>1</sup>, P. Rudan<sup>1</sup>.* 1) Institute of Anthropological Research, Zagreb, Croatia; 2) Department of Environmental Health, University of Cincinnati, Cincinnati, OH; 3) University of Zagreb, Zagreb, Croatia.

Anthropological data from the Croatian Adriatic islands ( $n = 66$ ) indicate that the island populations have remained considerably isolated from each other. To examine the effects of such isolation on patterns of gene diversity at microsatellite loci, we have studied the coefficient of gene differentiation ( $G_{st}$ ) between villages within islands as well as between islands using allele frequencies at 9 STR loci (D3S1358, vWA, FGA, THO1, TPOX, CSF1PO, D5S818, D13S317, and D7S820) in 31 villages of 4 islands (Hvar, Krk, Brac, and Korcula). Overall, none of the villages exhibits significant departure from Hardy-Weinberg expectations of genotype frequencies at these loci. Decomposition of heterozygosity ( $H$ ) as well as allele size variance ( $V$ ) indicated significant inter-island gene differentiation ( $G_{st}$  based on  $H = 0.003$ ,  $P < 10^{-4}$ ;  $G_{st}$  based on  $V = 0.004$ ,  $P = 0.007$ ), while the between villages within island component of genetic variation remain non-significant ( $G_{st}(H) = 0.003$ ,  $P = 0.073$ ;  $G_{st}(V) = 0.002$ ,  $P = 0.315$ ). The coefficient of coancestry  $q$  for the sampled unrelated individuals vary from village to village, ranging from 0 to 0.022, with an average of 0.010. Comparison of estimates of inbreeding coefficient ( $F$ ) based on genealogical data from these villages indicates that while  $q$  and  $F$  values are moderately correlated across villages,  $q$  is comparatively smaller (nearly one-half) than  $F$ . In other words, the high mutation rate at these STR loci diluted the effect of small effective size in producing correlation of alleles within individuals. The non-significant  $G_{st}$  between villages within the islands is an advantage for disease-gene association studies using microsatellite loci, since data from several villages within each island may be pooled to enlarge the sample size without introducing population substructure effects on the association results.

**LONG INTERSPERSED ELEMENTS (LINEs) AND THE GENETIC VARIABILITY OF NATIVE**

**AMERICANS AND OTHER RELATED POPULATIONS.** *L. Mateus Pereira*<sup>1, 2</sup>, *I. Fernandez*<sup>1</sup>, *A. Socorro*<sup>1</sup>, *M. Masleh*<sup>1</sup>, *G.D. Swergold*<sup>3</sup>, *M. Stoneking*<sup>4</sup>, *M.A. Batzer*<sup>5</sup>, *R.J. Herrera*<sup>1</sup>, *S.L. Bonatto*<sup>6</sup>, *F.M. Salzano*<sup>2</sup>. 1) Biological Sciences, Florida International University, Miami, FL; 2) Depto. Genetica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; 3) Department of Medicine, Columbia University, USA; 4) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany; 5) Department of Pathology, Louisiana State University Health Sciences Center, USA; 6) Inst. Biocincias, Pontificia Universidade Catlica do Rio Grande do Sul, Porto Alegre, RS, Brazil.

LINE-1 (L1) elements represent a large family of mammalian retrotransposons. Individuals with specific insertions are related by descent and have a known ancestral state, which is the absence of the insertion. The PCR assay employed is simple and utilizes two PCR reactions for genotyping each LID (LINE Insertion Dimorphism). The first amplification uses both 5' and 3' flanking unique sequence primers for the PCR. The second PCR contains a subfamily-specific primer and a 3' flanking unique sequence primer. In this study, we are characterizing the genetic diversity of polymorphic LINE elements (LIDs 1-6) in different South American Native populations, as well as, in other related human groups. So far, the samples include individuals from South America (Arhauaco, Toba, Guahibo, Karitiana, Suru, Lengua and Ayoreo); North America (Navajos) and Asia (Siberian Eskimo, Tuvinian, and Chinese). The frequency data for the LID1 insertion in these populations ranged from 0.15 for the Navajos to 0.48 for the Ayoreo. The data for LID5 in South American populations showed a high incidence of the occupied allele that varied from 0.7 for the Ayoreo to its complete fixation for the Karitiana. A comparison of the levels of heterozygosity for LID5 shows that it is lower than for LID1, since the presence of the insertion reached very high frequencies for this locus in those groups. So far, these two loci are showing a different pattern, and will be very interesting to see what the entire data is going to tell us about the genetic relationships among Native Americans and Asiatics in the peopling of the Americas scenario.

**Latitudes and the mc1r gene polymorphism among Asian populations.** *K. Nakayama*<sup>1</sup>, *R. Ootsuka*<sup>1</sup>, *A. Soemantri*<sup>2</sup>, *M.N. Isa*<sup>3</sup>, *S. Pookajorn*<sup>4</sup>, *I-H. Pan*<sup>5</sup>, *S. Harihara*<sup>1</sup>, *T. Ishida*<sup>1</sup>. 1) School Sci, Tokyo Univ; 2) School Med, Diponegoro Univ; 3) School Med, Univ.Sains Malaysia; 4) Faculty Arch, Silpakorn Univ; 5) School Med, Natl.Taiwan Univ.

Melanocortin 1 receptor (MC1R) is a key regulator of the mammalian melanogenesis. MC1R is coded by the mc1r gene that is only one identified gene accountable for the gradual human skin pigmentation found in geographically and ethnically different populations. We have studied diversity of the mc1r gene among 1012 individuals from a total of 29 Asian populations. PCR-direct sequencing, -SSCP, and -RFLP have been employed. We considered an African mc1r nucleotide sequence as the wild type. We identified nine nonsynonymous and two synonymous mutations including seven novel ones in this study. We found the absence of nonsynonymous mutations among heavily pigmented New Guinean Highlanders and the Veddas in Sri Lanka. This is consistent with the previous hypothesis that the mc1r gene has been under functional constraint to maintain substantial pigmentation against the high dose of ultra violet (UV) light in the equatorial regions and the mutated MC1R has been eliminated by purifying selection. Several Mongoloid groups living in high latitudes, showed an excess of nonsynonymous mutations. Our neutrality test has failed to detect the evidence of selection, however, this was comparable to the patterns of nonsynonymous mutations in the Europeans who are lightly pigmented. Light pigmentation in the Europeans is presumed as the result of weak UV light intensity that has relaxed the functional constraint against MC1R. We calculated  $q(s)$  of mc1r gene among subjected populations. The value showed a significantly positive correlation with latitudes (Kendall's  $\tau=0.371$ ,  $P<0.005$ ). It is indicated that selective force of UV light intensity, which is correlated with latitudes, generates geographical diversity of the mc1r gene; populations in higher latitude possess larger allelic diversity. These observations support (1) the existence of a universal distribution of the mc1r gene polymorphism and (2) the potentiality of the mc1r gene as a genetic basis for geographic adaptability of human skin pigmentation.

**Estimation of the Time to the Most Recent Common Ancestor by Tree-Partition.** *H. Tang*<sup>1</sup>, *D. Siegmund*<sup>1</sup>, *P. Shen*<sup>2</sup>, *P. Oefner*<sup>2</sup>, *M. Feldman*<sup>3</sup>. 1) Dept of Statistics, Stanford University, Stanford, CA; 2) Stanford Genome Technology Center, Stanford University, Stanford, CA; 3) Dept of Biological Sciences, Stanford University, Stanford, CA.

For a sample of DNA sequences that exhibits variation, a quantity of interest is the time to the most recent common ancestor of the sample, or TMRCA. When the sample is randomly taken from a population, the TMRCA provides information on the age of this population. If the sample consists of sequences sharing some other kind of unique event polymorphism (UEP), such as a disease mutation, the TMRCA represents a lower bound for the age of this mutation. We propose a statistical method of estimating the TMRCA of a sample of DNA sequences whose variation is characterized by single nucleotide polymorphisms (SNPs). Neither the proposed point estimate nor its confidence interval depends on the demographic history of the population, such as population size, growth, or migration pattern. Simulations show that the estimator is approximately unbiased, and that the confidence interval has approximately correct coverage probability in most situations. Performance of this estimator is compared with existing methods based on coalescence under the simplifying demographic assumptions that make the coalescent methods applicable. The new method is applied to SNPs on worldwide samples of human Y chromosome and mitochondrial DNA (mtDNA). The coalescence time for the extant Y chromosome population is estimated as 92,000 years (95% confidence interval: 61,000 to 129,000 years) corresponding time for the mtDNA population is much older at 226,000 years (95% confidence interval: 198,000 to 269,000 years).

**Patterns of genetic diversity at cytokine loci (*IL-13*, *IL-4*) in Andean populations: distinguishing the effects of selection and demographic history.** *E. Tarazona*<sup>1</sup>, *S. Fuselli*<sup>2</sup>, *D. Luiselli*<sup>2</sup>, *D. Pettener*<sup>2</sup>, *S.A. Tishkoff*<sup>1</sup>. 1) Department of Biology, University of Maryland, College Park, MD; 2) Unit of Anthropology. University of Bologna. Italy.

Since the end of the last glacial era, different parasites responsible for infectious diseases have constituted important selective pressures acting on the human genome. Because intra- and extra-cellular parasites activate immune responses of types Th1 and Th2, respectively, the regulatory genes involved in these pathways have been potentially targets of selection during human evolution. We have initiated a project to study genetic variability of cytokines associated with Th1 and Th2 immune responses in different human populations. Our primary aim is to understand how evolutionary forces acting on specific regions of the genome (selection, mutation, recombination) or on the whole genome (demographic history) could have shaped patterns of genetic diversity and linkage disequilibrium (LD). As a first step, we have studied three Peruvian native populations from the Andes, where infections by extra-cellular parasites are highly prevalent. We are examining genetic variation in genes that regulate the Th2 pathway, interleukin-4 (*IL-4*) and interleukin-13 (*IL-13*), and the extent of LD at different distances from these genes. We have tested several hypotheses of different types and levels of selection acting on these genes against a null hypothesis constructed using the patterns of variability of several neutral loci studied in these populations (Y-chromosome, mt-DNA, autosomal microsatellites). The analysis of these markers suggests that these populations of Andean farmers 1) conserve the signature of an ancient population expansion that likely occurred before the peopling of the Andes (20-25 ky ago) and 2) there is no evidence of drastic reductions in population size associated with the history of these populations. Comparison of genetic variability observed at *IL-4* and *IL-13* with these neutral markers allows us to discriminate among the evolutionary forces that have shaped the pattern of LD in this region and to characterize the signature of selection in the human genome. Funded by Burroughs Wellcome.

**HUMAN EVOLUTION AND BANTU MIGRATION: An Analysis of Alu insertion polymorphisms. M.C.**

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Alu elements comprise the most numerous family of short interspersed repetitive elements (SINEs) in the human genome. Alu elements are thought to be derived from the signal recognition particle 7SL RNA gene. Most Alu elements are around 300 nucleotides (nt) in length and are present in 500,000 copies per haploid genome. Alu insertion polymorphisms are excellent markers for human evolutionary studies because of their properties including being stable mutation events for which the ancestral state is known to be the absence of the Alu element. In this study, a polymerase chain reaction (PCR) assay was used to examine the frequency of Alu insertions in polymorphic loci. These Alu elements were used to analyze unrelated individuals in different populations outside and inside of the African continent to follow the migration that happened during the period of history known as Bantu expansion. This was one of the largest migrations beginning approximately 1000 BC that continued into the nineteenth century of the Christian era with profound effects on the history of subequatorial Africa. The Bantu spread from Nigeria and Cameroon toward the east and south started during the Iron Age, in Neolithic times (around 1000 B.C). The phylogenetics analysis of these Alu insertion polymorphisms supports the theory that the Bantu migration arose in the Central West Coast of Africa and spread to the west, southwest and southern coast of the African continent.



**Signature of selection at the G6PD locus inferred from patterns of nucleotide variation and linkage**

**disequilibrium in Africans.** *B.C. Verrelli<sup>1</sup>, G. Argyropoulos<sup>2</sup>, G. Destro-Bisol<sup>3</sup>, S.M. Williams<sup>4</sup>, S.A. Tishkoff<sup>1</sup>.* 1) Biology, Univ Maryland, College Park, MD; 2) Pennington Biomed Res Center, Baton Rouge, LSU, LA; 3) Anim/Hum Biol, "La Sapienza", Italy; 4) MeHarry Med College, Nashville, TN.

Mutations within the glucose-6-phosphate dehydrogenase (G6PD) gene that result in reduced enzyme activity affect more than 400 million people and are responsible for a number of haemopathologies. G6PD deficiencies have also been implicated in malarial resistance and more than 130 coding-sequence mutations have been identified. The most common alleles in Africa are B (normal enzyme activity), A (85% activity), and A- (12% activity), which is strongly correlated with malarial endemicity. A recent study utilizing both SNPs within the G6PD gene (Xq28) and microsatellites within close proximity found both strong linkage disequilibrium (LD) and a unique haplotype structure associated with the B/A/A- polymorphism that indicates a recent origin and rapid increase in frequency of the A- allele, likely associated with the spread of malarial infection (Tishkoff et al., *Science*, in press, 2001). Our current study consists of DNA sequence data for 5kb of the G6PD gene from Africans (n=75) and compares it to samples of non-Africans (n=25) and chimpanzees (n=4). Our sample of B, A, and A- alleles reveals several previously uncharacterized SNPs. Although the ancestral B allele dominates in frequency, B haplotypes possess significantly less SNP variation than A/A- haplotypes, suggesting that the A allele was historically greater in frequency and that the B allele has recently increased in populations, possibly due to positive selection. Comparative analysis with the chimpanzee data also shows a pronounced split of the B and A/A- haplotypes into two distinct clades implying balancing selection. The pattern of sequence variation associated with the three common G6PD alleles not only confirms the recent and rapid increase of the A- allele, but also indicates that the A/B polymorphism has had both an ancient and recent evolutionary impact on human populations. Additionally, in an effort to further characterize the signature of selection in the human genome, we investigated LD at various distances from the G6PD gene.

**Y chromosome polymorphisms indicate an ancient migration from the Himalayas to Japan.** *B. Su<sup>1,2</sup>, G.V. Ramana<sup>3</sup>, S.H. Lu<sup>3</sup>, B. Wen<sup>4</sup>, R. Deka<sup>1</sup>, P. Underhill<sup>5</sup>, R. Chakraborty<sup>1</sup>, L. Jin<sup>1,4</sup>.* 1) Dept of Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Laboratory of Comparative Genomics, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, China; 3) Human Genetics Center, University of Texas-Houston, Houston, TX; 4) Institute of Genetics, School of Life Sciences, Fudan University and Morgan-Tan International Center for Life Sciences, Shanghai, China; 5) Department of Genetics, Stanford University, Stanford, CA.

The Alu insertion polymorphism on the Y chromosome (YAP+) has a characteristic distribution in worldwide populations, mainly restricted in Africa, Central Asia and East Asia. The ongoing controversy on the origin of YAP+ and its mysterious occurrence in East Asia, i.e. dominant in Tibetan and Japanese populations but basically absent in other East Asian populations call for further studies along this ancient human lineage. Here we report our preliminary result on a systematic screening of YAP+ in more than 2,500 male individuals from 70 world populations. Nine Y chromosome biallelic markers derived from the YAP+ polymorphism and seven Y chromosome micro satellites were typed in 172 YAP+ individuals from Africa (35), South Asia (9), Southeast Asia (12) and East Asia (116). Our results showed that African and Asian YAP+ are distinctive from each other due to a deep genetic divergence. No evidence was found to support the hypothesis of an Asian origin of YAP+. Among the East Asian YAP+, Tibetans are the most diversified population and have the haplotype ancestral to those in Japanese, indicating an ancient migration from the Himalayas to Japan. In addition, a relatively recent migration of YAP+ from Tibet to Yunnan (southwestern China) was also implied by the homogenous YAP+ haplotypes in Yunnan ethnic populations, which was signified by the low microsatellite diversity and an almost fixed unique microsatellite allele at locus DYS392 in Yunnan populations.

**The effects of positive selection on the frequency spectrum of mutations and on patterns of linkage disequilibrium.** *M.F. Przeworski*. Statistics, Oxford Univ, Oxford, England.

Considerable debate has focused on what proportion of changes fixed between species are favorable, as well as what types of substitutions are most likely to have been selected. Answers to these questions will help to understand the genetic basis of adaptation and may also aid in the identification of regions of functional importance. A mode of selection of particular interest is a selective sweep, in which a strongly favored variant arises and quickly increases in frequency until it fixes in the population. Here, I examine coalescent models of a single sweep and of recurrent (but non-overlapping) sweeps. The first model is salient to loci where there is independent evidence of recent selection while the second might be more appropriate for randomly chosen loci. I examine the effects of these types of positive selection on pairwise measures of linkage disequilibrium as well as on estimates of the population recombination rate. I also consider the power of "tests of neutrality" to detect the effects of selective sweeps.

**Sequence Variations of Interleukin-1 receptor antagonist (IL-1Ra) gene in humans and non-human primates.**

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Interleukin-1 (IL-1) is a major mediator of inflammation and exerts pleiotropic effects on immune system. In the process of rheumatism, it is one of the pivotal cytokines in initiating disease. Interleukin-1 receptor antagonist, a natural modulator existing in the body, can bind IL-1 receptor and block its effects. To investigate genetic variations of this modulator in humans and non-human primates, the IL-1Ra was sequenced covering all the six exons of this gene, in 12 humans (*Homo sapiens*), 11 chimpanzees (*Pan troglodytes*), 4 gorillas (*Gorilla gorilla*), 2 orangutans (*Pongo pygmaeus*), 1 siamang (*Hylobates syndactylus*) and 1 rhesus monkey (*Macaca mulatta*). A total of 3,873 bp DNA sequence was obtained including 1925 bp in the exons and 1948 in the non-coding regions. There are 338 variant sites across species including 34 in the exons, 197 in the introns and 107 in the 5'/3' flanking regions. In the exons, two synonymous sites were observed in humans, 1 in chimpanzees and 1 in gorillas. Two amino acid changes exist between human and chimpanzee/gorilla and no non-synonymous changes were detected within species. In the non-coding regions, 18 variable sites exist in humans, 24 in chimpanzees and 5 in gorillas. Neutrality test didn't indicate any deviation from the neutral model.

**mtDNA sequence diversity in Antioquia (Colombia).** *A. Ruiz-Linares*<sup>1,2</sup>, *I. Soto*<sup>2</sup>, *C. Duque*<sup>2</sup>, *J. Ospina*<sup>2,3</sup>, *G. Bedoya*<sup>2</sup>, *L.G. Carvajal-Carmona*<sup>1,2</sup>. 1) The Galton Laboratory, Department of Biology, University College London, UK; 2) Laboratorio de Genetica Molecular, Facultad de Medicina, Universidad de Antioquia, Colombia; 3) Departamento de Psiquiatria, Facultad de Medicina, Universidad de Antioquia, Colombia.

We recently used Y-chromosome and mtDNA markers to assess the ethnic background of the population of Antioquia in North West Colombia. This population has a history of isolation and since its founding in the 16th-17th centuries has expanded over a thousand times (to a current size of about 3-4 million). A Native American ancestry was detected in about 1% of Y-chromosomes and in around 90% of mtDNA lineages indicating that it was mostly native women and immigrant men that founded Antioquia. To assess more fully the female ancestry of this region we sequenced mtDNA hypervariable segment I in 85 individuals with confirmed Antioquian ancestry. Of these, 74 carry variants characteristics of the major founder Amerind lineages: 40 belong to haplogroup A, 28 to haplogroup B, 5 to haplogroup C and 1 to haplogroup D. Among the 11 non-Amerind sequences identified 2 correspond to European lineages while 9 have an African ancestry. Consistent with the high frequency of Native American mtDNA lineages in Antioquia, the mitochondrial diversity in the province is similar to that of Amerind populations and is significantly lower than in most European populations. Antioquian Amerind sequence diversity is consistent with a constant population size ( $Tajima's D = -0.58$   $P > 0.05$ ;  $F_u$  vs  $F_s = -4.66$   $P > 0.05$ ), the distribution of pair-wise sequence mismatches showing two distinct peaks at 0 and 6 mismatches. Most human mtDNA sequence data show evidence of a Pleistocene demographic expansion with the absence of such a signal having been detected in hunter-gatherers and in some Native American populations. The absence of a signal of population expansion in Antioquia and in some Native Americans could relate to the Native population collapse caused by the Spanish colonization. This pattern could also be influenced by the strong drift affecting many Native American populations, particularly in South America.

**The Dopamine Beta-Hydroxylase C-1021T Polymorphism: Evolutionary Origins and Relationship to Plasma DBH Activity in Nonhuman Primates.** *C.P. Zabetian<sup>1,3</sup>, G.M. Anderson<sup>2</sup>, R. Hoppenot<sup>3</sup>, J.R. Kaplan<sup>4</sup>, J.F.*

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Dopamine beta-hydroxylase catalyzes the conversion of dopamine to norepinephrine. Human plasma DBH enzyme activity levels vary over 100-fold between individuals. We have recently identified a novel, putative functional polymorphism (C-1021T) in the 5' upstream region of the DBH gene that accounts for 35-52% of the total variation in activity in samples from African-American, European-American, and Japanese populations. Individuals homozygous for the minor T allele have very low activity levels. A previous study of nonhuman primates revealed levels of variation in plasma DBH activity comparable to that found in humans. In this study, we examined the evolutionary history and relationship to phenotype of the DBH C-1021T polymorphism in nonhuman primates. We obtained plasma and DNA samples from great apes (*Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla*, *Pongo pygmaeus*), gibbons (*Hylobates* spp.), old world monkeys (*Papio* spp., *Macaca* spp.), and new world monkeys (*Saimiri sciureus*). Preliminary sequencing and genotyping results indicate that the T allele is ancestral, and that the C allele arose at some point between the divergence of orangutans and gorillas from the chimpanzee-human lineage. All chimpanzee (*Pan troglodytes*, *Pan paniscus*) and gorilla samples studied to date are homozygous for the C allele. Collection of additional samples is ongoing to determine if the C-1021T polymorphism exists in hominoid species other than humans, and if so, whether a close association exists between genotype and plasma DBH activity. Supported by: USDVA, NIDA, NARSAD.

**The Acadian USH1C 9-repeat VNTR(t,t) allele arose independently of the 3-repeat VNTR(t,t) allele.** *S. Savas*<sup>1</sup>, *S.S Ng*<sup>1</sup>, *P.L. Deininger*<sup>2</sup>, *M.A. Batzer*<sup>3</sup>, *B.J.B Keats*<sup>1</sup>. 1) Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA; 2) Tulane Cancer Center, Department of Environmental Health Sciences, New Orleans, LA; 3) Louisiana State University Biological Computation and Visualization Center, Baton Rouge, LA, USA.

A 45 bp VNTR was identified in intron 5 of the Usher syndrome type IC gene (USH1C). The 9-repeat VNTR allele is in complete linkage disequilibrium with the USH1C mutation in the Acadian population, and is characterized by the presence of two repeat units at the 3' end that contain a T at the eighth nucleotide position rather than a G (VNTR(t,t)). This VNTR does not display meiotic or mitotic instability, and the 9-repeat VNTR(t,t) allele is found solely in Acadian USH1C patients. We analyzed 152 unrelated individuals (32 Acadian, 20 African, 48 European, 52 Asian) with repeat sizes ranging from 2 to 8, and found that one Acadian and three European individuals were heterozygous for a 3-repeat VNTR(t,t) allele. All other alleles in this sample had only one repeat unit at the 3' end with a T (VNTR(t)). Additionally, among the parents of Acadian USH1C patients, we found one who had the 3-repeat VNTR(t,t) allele and the 9-repeat VNTR(t,t), demonstrating that homozygosity for the VNTR(t,t) structure is not sufficient to cause USH1C. In order to gain insight into the origin of the 9-repeat VNTR(t,t) allele, we obtained haplotypes at 11 SNPs in close proximity to the VNTR locus for the chromosomes containing the VNTR(t,t) alleles, and found that the 3-repeat VNTR(t,t) haplotype was quite different from the 9-repeat Acadian VNTR(t,t) haplotype. These results indicate that the 9-repeat VNTR(t,t) allele arose independently of the 3-repeat VNTR(t,t). Moreover, we propose that unequal crossing-over is a likely explanation for the differently sized VNTR(t) alleles as well as the rare 3- and 9-repeat VNTR(t,t) alleles.

**Regression trees to select maximally-linked phenotypes in relative-pair linkage analysis of multivariate traits.**

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We have developed a nonparametric relative-pair linkage analysis method appropriate for highly multivariate phenotypic data. This method adaptively finds subsets of phenotypes that demonstrate the strongest evidence for linkage. In each family and for a particular locus, the difference between observed and expected identical-by-descent (IBD) allele sharing is calculated for all pairs of individuals informative for linkage. Then, classification and regression trees are used to develop predictions for these IBD differences using pair-level covariates based on the phenotypes. Hence, we can identify subsets of pairs with high mean levels of allele sharing. Bootstrapping can be used to stabilize cutpoint selection, and cross-validation optimizes the size of the regression tree. The tree predictions are then used to identify successive subsets of pairs among whom the evidence for linkage is measured.

We applied our method to 414 pairs of individuals from 68 multiplex families ascertained for asthma. A regression tree was grown using IBD estimates at a candidate chromosomal region. After cross-validation, the regression tree identified no phenotypic traits that were strongly associated with increased allele sharing. However, to illustrate our algorithm, we chose a tree with 5 terminal nodes. In 132 pairs, who had either low asthma severity scores and low IgE or high atopic indices, a non-parametric linkage score of 3.32 was obtained. The significance of this test for linkage will be evaluated by simulation.

This approach can be used to filter high-dimensional phenotypic data. For example, gene-gene interactions might be identified by including mutation information at known candidate genes among the phenotypic data. However, due to the adaptive nature of the algorithm, results must be interpreted cautiously and validated in independent data.



**Linkage simulation enhances decision making strategies for study of lung cancer (LC) pedigrees.** *M.de Andrade*<sup>1</sup>, *J. Slusser*<sup>1</sup>, *C.I. Amos*<sup>2</sup>, *J. Bailey-Wilson*<sup>3</sup>, *P. Fain*<sup>4</sup>, *M.W. Anderson*<sup>5</sup>, *G. Petersen*<sup>1</sup>. 1) Health Sciences Research, Mayo Clinic, Rochester, MN; 2) Epidemiology, UT M.D. Anderson Cancer Center, Houston, TX; 3) NGHRI, Bethesda, MD; 4) University of Colorado, CO; 5) University of Cincinnati, OH.

As the Genetic Epidemiology of Lung Cancer Consortium identifies high risk LC families, a challenge has been in deciding which families/individuals to study, given high sporadic rate and difficulty of accrual. **Objective:** To assess the value of linkage simulation for data collection as a way to inform data collection and fine mapping of high risk LC pedigrees. **Methods:** One thousand replicates of a 40 member multiplex genotyped LC pedigree were simulated using SLINK and GENEHUNTER, compared to the observed NPL score (1.7) with a 13-allele marker. We evaluated maximum information in the pedigree that could be obtained with fine mapping. **Results:** Bimodal distribution of expected NPL scores were observed with this pedigree structure. Compared to the observed NPL score, the pedigree was found to contain additional power to detect linkage by fine mapping. The inclusion of a newly diagnosed affected LC individual will give sufficient power to detect linkage. **Conclusions:** Linkage simulation of individual pedigrees can enhance decision-making in LC mapping studies. Following genome scanning, simulation studies of the power to detect linkage by fine mapping in specific families may direct data collection strategies.

**Combined Linkage and Association Interval Mapping of Quantitative Trait Loci.** *R. Fan<sup>1</sup>, M. Xiong<sup>2</sup>*. 1) Dept Health Evaluation Sciences, 600 Centerview Drive, Pennsylvania State Univ, Hershey, PA 17033; 2) Human Genetics Center, University of Texas-Houston, P.O. Box 20334, Houston, Texas 77225.

Two methods, linkage analysis and linkage disequilibrium mapping, are usually utilized for mapping quantitative trait loci (QTL). Both linkage analysis and linkage disequilibrium mapping have their advantages and disadvantages. Using only one marker, one may carry out linkage analysis. Using two or more markers, it is possible to flank the QTL by interval mapping. In this report, we propose joint linkage and linkage disequilibrium interval mapping of QTL by considering likelihoods which utilizes flanking marker informations and includes both recombination fractions and linkage disequilibrium coefficients as parameters. We carry out the analysis of model building, parameter estimations and power calculations to show the advantages of the method: (1) both population and family pedigree data can be used simultaneously; (2) population data can provide information for linkage disequilibrium mapping, and family pedigree data can provide information for both linkage analysis and linkage disequilibrium mapping; (3) interval mapping can provide fine mapping for the location of QTL.

**An adaptive test procedure for linkage analysis of quantitative traits.** *E.I. Drigalenko.* Neuropsychiatry & Behavior Sci, Texas Tech Univ Health Sci Ctr, Lubbock, TX.

A method of linkage analysis for a binary trait (a complex disease) is described that is based on sib pairs and takes into account the mode of inheritance of the trait. Under assumptions that the trait is determined by one two-allele locus, Hardy-Weinberg equilibrium, and no allelic association, the likelihood function is derived as a function of variance components. It is shown that the previously proposed affected sib pair tests comprise a family of studentized efficient score statistics. They are based on a hidden parameter, the recessive angle, which describes the mode of inheritance of the trait. Its extreme values give the two commonly used two-allele and mean test statistics. An environmental factor has been taken into account. The empirical distribution function is advocated to evaluate the significance level of the test. The proposed method is distribution-free, the locally most powerful against small values of the genetic variances, based on the distribution function under the null hypothesis, and controls the type I error even if the trait parameters are misspecified.

**The genealogy of Fanconi Anaemia patients homozygotic for the type I and type II Afrikaner mutations. S.**

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Fanconi anaemia (FA), a rare autosomal recessive genetic disorder, is known to occur at a higher than normal incidence among the white Afrikaans speaking population of South Africa. The disease is characterised by progressive bone marrow failure, starting predominantly in childhood, various phenotypic abnormalities as well as an increased sensitivity to the clastogenic agents diepoxybutane (DEB) or mytomycin C (MMC), resulting in chromosomal aberrations. Initial genealogical investigations on 12 families affected by FA but with unknown mutational status, identified Guillaume Nel and/or his spouse Jeanne de la Batt as common ancestors. A founder effect, the cause of the high incidence of FA among this population group, was further substantiated by results obtained through complementation studies and mutation screening, showing the predominance of complementation group A and mutation types I, II and III. The existence of three mutation types among the descendants of Guillaume Nel and Jeanne de la Batt raised the possibility of more than one founder pair or family being involved. For this reason an additional genealogical investigation was carried out, limited to seven and six FA parents carrying respectively the Afrikaner types I and II mutations. Apart from GN/JDLB, HDP and wife CD also featured prominently as possible founders for the FANCA Afrikaner type I mutation whereas either PV or MDP are candidate founders of the FANCA Afrikaner type II mutation.

**General Tests for Departures from Hardy-Weinberg Proportions with Pedigree Data.** *D.J. Schaid, S.K. McDonnell, J.M. Cunningham, B.J. Peterson, S.N. Thibodeau.* Mayo Clinic, Rochester, MN.

When performing genome scans for linkage of genetic markers with traits, it is critical to eliminate genotyping errors, which can dramatically reduce the evidence for linkage. In addition to evaluating Mendelian transmission of markers, the quality of markers can be evaluated by testing whether the marker genotype frequencies adhere to Hardy-Weinberg proportions (HWP), because an excess number of homozygotes can indicate undetected alleles. The common tests for HWP require independent subjects, to avoid an inflated rate of false claims. When analyzing pedigree data, the independent founders of the pedigrees can be used, yet they are frequently not available for the study of older-onset disease. Two approaches have been used to circumvent this problem. A common approach is to randomly sample one subject per pedigree. However, this approach is inefficient, and has the potential to miss problem genotypes simply due to the random sampling. A second approach depends on a probability model for the genotypes, and uses residual deviations from the fit of the model to account for correlated data. This approach can be inefficient because it does not directly account for the different types of relationships among pedigree members. We propose new methods to test for departures from HWP with pedigree data that use all study subjects while accounting for dependencies among relatives. The methods are general enough to consider a variety of departures, using different weighting schemes, while reducing the degrees of freedom in order improve power. For example, one weighting scheme is sensitive to departures due to an excessive number of rare homozygotes. In addition to the global tests for departure from HWP, we propose pedigree diagnostic statistics to examine the influence of each pedigree. These diagnostics can be useful to identify problem pedigrees. Our new methods are applied to our ongoing genome-wide linkage study of hereditary prostate cancer to illustrate the benefits of this type of statistical quality control.

**A novel meta-analysis method which corrects for publication bias.** *G.P. Sreekumar<sup>1</sup>, D.V. Zaykin<sup>1</sup>, L.A. Zhivotovsky<sup>2</sup>*. 1) Department of Population Genetics, GlaxoSmithKline Inc., Research Triangle Park, NC; 2) Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia.

Recent interest in the genetics of common complex diseases has resulted in numerous linkage and or association studies. The goal of these studies is to find the disease susceptibility genes, which confers risk for the disease (which may in fact be very small, considering the complexity of these diseases). Common methods combine such information through meta-analytic approaches, for example by evaluating the distribution of sum or a product of p-values (sometimes transformed), often weighted by the size of the studies. The major flaw of this approach is that the probability of a particular study being published is positively correlated with the significance of the obtained result and thus the combined estimate is likely to be largely inflated. Here we provide a method that is robust against the publication bias and that allows a test for the presence of association between genetic polymorphisms and the disease. The method explores the fact that the distribution of significant p-values depends on the presence of the actual effect.

Asthma is one of the common chronic childhood diseases in developed nations. Mapping of susceptibility loci for asthma is hampered by high population frequency, incomplete penetrance, phenocopies and genetic heterogeneity. Although significant progress has been made in the mapping of asthma susceptibility loci in the past several years, even large studies have low power to map genes of modest effect. We applied our meta-analysis technique to several candidate gene studies published in the literature and found that the following candidate genes were found to be significantly associated with asthma. Fc epsilon receptor beta (p-value=1e-05), Interleukin 4 receptor (p-value=0.01), Interleukin 4 (p-value=2e-05), Tumor necrosis factor (p-value=7e-05), and Angiotensin converting enzyme (p-value=0.05).

**Concordances between self-reported disease and laboratory results of family members participating in a genetic family study of diabetic nephropathy. Results from the San Antonio Center of the multi-center FIND. R.**

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The Family Investigation of Nephropathy and Diabetes (FIND) searches for diabetic nephropathy (DN) genes. As one of 8 centers, we have enrolled 40 Mexican American families through a proband with end stage renal disease (ESRD) since December 2000. Each family has on average 2.5 members (range:1-9). We received lab results from 100 subjects: 40 probands, 60 relatives (58 siblings; 2 parents). From 13 families, we currently have only probands' lab results.

Of the 40 probands, 19 were females; average age was 56.2 years. Average age of onset for diabetes was 34 years (range:17-55), and 53.3 years for ESRD (range:35-68). On average, probands had 19.7 years diabetes before receiving dialysis. 91.9% had diabetes <sup>3</sup>9 years before needing dialysis.

Relatives were on average 56.3 years old (range:32-87), 72% were females. 34 relatives reported diabetes, 3 reported ESRD. Family members were tested for diabetes (HbA1C) and kidney function (urine albumin/creatinine and protein/creatinine, serum creatinine, BUN). Lab tests showed that 18 of the 34 diabetic relatives had microalbuminuria or proteinuria. 26 relatives reported not having diabetes. 12 had HbA1C<sup>3</sup>6.0 (range: 6-13). 5 had HbA1C<sup>3</sup>7.0 and were classified as diabetics; 2 of them had microalbuminuria or proteinuria. 5 subjects with 6.0£HbA1C<7.0 had microalbuminuria or proteinuria. Among the 14 relatives with HbA1C<6.0, 3 had microalbuminuria or proteinuria.

We will present (1) the goal of our center in the FIND, (2) updated information about the probands and the concordances between self-reports and lab results, and (3) correlate lab results with their currently collected self-reports about their kidney status.

This research has been funded by the NIDDK/NIH (DK57295) and the San Antonio Area Foundation.

Program Nr: 1258 from the 2001 ASHG Annual Meeting

**Liability Groups in Linkage Analysis.** *S.S. Shete, C.I. Amos.* Dept Epidemiology, Univ Texas MD Anderson CA Ctr, Houston, TX.

In this note we present a way of incorporating individual specific liability classes for linkage analysis. In linkage studies, we may have information about different covariates. In this method, one uses the best fitting model obtained by segregation analysis. This model will give parameter estimates such as familial effects, covariate effects, age of onset effects, and susceptibility. Here we show how these estimates can be used to form individual specific liability classes and how one can use standard linkage analysis program LINKAGE to perform such analysis.



**Strategies for SNP Selection for Linkage Disequilibrium Mapping.** *A. Aelony, S. Lewitzky, K. Yu, A. Parker, J. Meyer, R. Martin.* Dept Human Genetics, Millennium Pharmaceuticals Inc, Cambridge, MA.

Recent advances involving the Human Genome Project and associated genomics consortia have populated public databases with an abundance of information on Single Nucleotide Polymorphisms (SNPs) in the form of a dense genome-wide map. Despite this new wealth of SNP information, many questions remain regarding the impact of SNP selection on power to detect linkage disequilibrium (LD) in fine mapping studies. In the analysis of a single SNP, we explore the impact of allele frequency on power. We then proceed to more realistic scenarios where multiple SNPs in a small region are analyzed, to determine how combinations of allele frequencies can be chosen to optimize power. We simulate nuclear family data with population parameters based on values observed in a study of schizophrenia in a Finnish population. For specified disease models, we compare power of single-point TDT-based LD methods among different SNP selection strategies. Results are controlled for multiple testing, taking into account background LD using recently developed Monte Carlo methods.

**Characterization of Marker-Marker Linkage Disequilibrium in a Finnish Founder Population.** *M. Harmon*<sup>1</sup>, *K. Rinard*<sup>1</sup>, *G. Barnes*<sup>1</sup>, *A. Foti*<sup>1</sup>, *C. Reeves*<sup>1</sup>, *G. Chan*<sup>1</sup>, *A. Parker*<sup>1</sup>, *J. Lonquist*<sup>2</sup>, *T. Paunio*<sup>2</sup>, *C. Richard*<sup>4</sup>, *L. Peltonen*<sup>2,3</sup>, *J. Meyer*<sup>1</sup>, *R. Martin*<sup>1</sup>. 1) Human Genetics, Millennium Pharmaceuticals, Cambridge, MA; 2) National Public Health Institute, Helsinki, Finland; 3) Human Genetics, UCLA School of Medicine, Los Angeles, CA; 4) Genetics Institute, Wyeth-Ayerst Research, Cambridge, MA.

As part of a study of schizophrenia in a Finnish population, we are fine mapping a 35cM region containing a possible disease susceptibility locus or loci on chromosome 1q32-42. A total of 216 microsatellite and 50 SNP markers are typed in two independent samples. One sample drawn from the general population of Finland consists of 278 nuclear pedigrees; the second is from an internal isolate in the northeast of Finland and is made up of 168 nuclear pedigrees, 122 of which can be linked into one large pedigree. Average spacing of microsatellites is 150 kb apart, however markers are non-uniformly distributed: 40% are spaced  $\leq 10$  kb apart, 30% between 10 and 100 kb, and another 30% between 100 kb and 1 Mb. We are investigating the extent of linkage disequilibrium (LD) in our experimental data and how it varies over this 35cM region. Several LD coefficients including Lewontin's  $D'$  are calculated from founder haplotypes, estimated from pedigree data using Simwalk, by performing multiple independent runs and pooling estimates. Preliminary results for SNPs looking at 332 chromosomes in 83 pedigrees (with both parents typed) from the general Finnish population sample indicate that LD with  $D'$  of at least 0.6 extends up to 150 kb. As SNPs tend to vary according to mutability and heterozygosity when compared with microsatellites we also compare LD estimates in subintervals containing both marker types to determine whether LD estimates may differ depending upon the type of marker used. Comparisons are also made across samples to look at how historic population bottlenecks may affect levels of LD. Finally, to assess the affect of varying amounts of missing genotype data on haplotype estimates and subsequently the LD coefficients based upon them, we stratified pedigrees according to the number of typed parents and compared the resulting LD measures.

**Genetic associations between pubertal growth and skeletal maturity in healthy boys and girls.** *B. Towne<sup>1</sup>, S.A. Czerwinski<sup>1</sup>, E.W. Demerath<sup>1</sup>, A.F. Roche<sup>1</sup>, J. Blangero<sup>2</sup>, J.S. Parks<sup>3</sup>, M.R. Brown<sup>3</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.

A number of single gene defects result in disturbances of both skeletal growth and skeletal maturation, but little is known of the nature of shared genetic influences on skeletal growth and maturation during normal childhood development. In this study, we examined the genetic architecture of skeletal growth and maturation during the pubertal growth spurt. Individual growth curves were fitted to serial stature data from 158 boys and 205 girls in 105 families in the Fels Longitudinal Study using the triple logistic method in the program AUXAL (Bock et al., 1994). Each individual's age (AGE) at minimum height velocity (MHV) before puberty, and at peak height velocity (PHV) during puberty, were obtained, along with their height (HT) and growth velocity (GV) at those ages. These 363 boys and girls also had hand-wrist radiographs taken within one year before and after the timing of their MHV and PHV from which skeletal age (SA) assessments were made using the FELS method (Roche et al., 1988). Estimates of SA at MHV and SA at PHV were derived by interpolation. The heritability of each trait was estimated using SOLAR (Almasy and Blangero, 1998); a modified version of SOLAR was used to estimate genetic correlations between SA at MHV, and SA at PHV, and the other traits. The heritabilities were: AGE at MHV = 0.82, HT at MHV = 0.61, GV at MHV = 0.72, SA at MHV = 0.67, AGE at PHV = 0.82, HT at PHV = 0.59, GV at PHV = 0.59, and SA at PHV = 0.56. The genetic correlations were: SA at MHV & AGE at MHV = 0.53, SA at MHV & HT at MHV = 0.53, SA at MHV & GV at MHV = -0.36, SA at PHV & AGE at PHV = 0.36, SA at PHV & HT at PHV = (0), and SA at PHV & GV at PHV = -0.55. Overall, these findings reveal: 1) highly significant heritabilities of different parameters of physical growth in stature, and of skeletal maturity, during puberty; and 2) incomplete (or no) pleiotropic effects of genes on parameters of physical growth in stature and skeletal maturity during puberty. Supported by NIH grants HD12252, HD36342, and MH59490.

**Founder effects and the peopling of the New World.** *S. Bourgeois*<sup>1</sup>, *E. Zietkiewicz*<sup>1</sup>, *V. Yotova*<sup>1</sup>, *R. Michalski*<sup>3</sup>, *A. Ruiz-Linares*<sup>4</sup>, *D. Labuda*<sup>1,2</sup>. 1) Research Center, Hopital Sainte-Justine, and; 2) Pediatrics Department, University of Montreal, Montreal, QC, Canada; 3) Victoria Hospital, Prince Albert, SK, Canada; 4) The Galton Laboratory, University College, London, United Kingdom.

Genetic studies of maternally inherited mitochondrial DNA and paternally inherited Y-chromosome do not provide clear distinction between the three linguistic groups of the American populations believed to represent three independent waves of the New World colonization through Beringia. Here, we present the data on a nuclear, X-linked marker studied in NaDene and Paleoindian populations. *Dys44* haplotypes consist of 35 segregating sites found within 8 kb of the intronic DNA flanking exon 44 of the dystrophin gene on Xp21. Out of the ten major haplotype families identified in a worldwide sample (n=1315) of the X-chromosomes, three are of particular interest. The haplotype families B002 and B005 with the overall worldwide frequencies of 23% and 6.5%, respectively, appear to be virtually absent in the populations from Northern America (NaDene and Ojibwa), as well as from Central (Maya) and Southern America (Karitiana). This finding provides an argument for a single founder effect that includes both Paleoindian and NaDene speakers. On the other hand, the frequency of the haplotype family B004 is dramatically increased in Maya (32%) and even more so in Karitiana (69%) , compared to 3.3% in Ojibwa and 7.5% in NaDene. This suggests local founder effects related to the colonization of Central and Southern America, but not correlated with the major linguistic subdivisions. Additional data that are currently being collected on the *dys44* diversity in American populations will be presented in correlation with the existing evidence of mitochondrial and Y-chromosome diversity. The present evidence points to a single major colonization event and subsequent diversification of the population of the New World. (Supported by CIHR; S.B. has a studentship of HSJ Research Center).

**The HGDP-CEPH genome diversity cell line panel.** *H.M. Cann*<sup>1</sup>, *C. De Toma*<sup>1</sup>, *A. Marcadet-Troton*<sup>1</sup>, *J. Dausset*<sup>1</sup>, *G. Thomas*<sup>1</sup>, *H.T. Greely*<sup>2,3</sup>, *L.L. Cavalli-Sforza*<sup>3</sup>. 1) Foundation Jean Dausset, CEPH, Paris, France; 2) Law School, Stanford University, Stanford, CA; 3) Department of Genetics, Stanford University School Medicine, Stanford, CA.

Human genome diversity studies, involving the typing of various populations with polymorphic markers, provide information concerning their genetic histories and the extent of polymorphism and, relevant for mapping genes for complex traits, about linkage disequilibrium in their respective genomes. The Human Genome Diversity Project (HGDP) and the *Centre d'Etude du Polymorphisme Humain* (CEPH) have undertaken a collaboration to collect and produce lymphoblastoid cell lines (LCLs) from populations throughout the world in order to promote and coordinate genome diversity studies. An initial phase of the collaboration is now completed: 1066, already-existing, mycoplasma-free LCLs from various populations and milligram quantities of corresponding high quality DNAs are banked at CEPH. These LCLs originate, for the most part, from indigenous groups in sub-Saharan and north Africa; west, east, central, north and southeast Asia; New Guinea and Melanesia; Europe and the Americas. In general, some 50 populations are represented.

DNA from these cell lines will be distributed to investigators who are ready to collaborate with HGDP-CEPH by typing DNA from all LCLs with their markers and contribute the typing results to a central database. The DNAs will also be typed with a subset of 400 well-mapped STRs from the Marshfield collection (Dr. J. Weber). Those investigators who undertake resequencing studies of relatively large genome regions need not necessarily sequence DNAs from all the panel populations nor from all LCLs within a population. Cell lines will not be distributed.

Identifying information for each LCL is limited to the sex and population and geographic origin of the sampled individual. Local ID numbers of LCLs received at CEPH have been transformed to different code in order to preserve subject identity. The informed consent practices used for taking the original blood specimens, many obtained 10-20 years ago for production of these cell lines, are being evaluated.

**Survey of Ashkenazi Jewish SNPs in a 471 kb region of chromosome 9q31 as compared to the public SNP database (dbSNP).** *S.P. Gill<sup>1</sup>, M. Leyne<sup>1</sup>, J. Mull<sup>1</sup>, M.P. Cuajungco<sup>1</sup>, C.M. Robbins<sup>2</sup>, I. Makalowska<sup>2</sup>, A. Blumenfeld<sup>3</sup>, M. Brownstein<sup>2</sup>, J.F. Gusella<sup>1</sup>, S.A. Slaugenhaupt<sup>1</sup>.* 1) Harvard Inst Human Genetics, Mass General Hosp, Boston, MA; 2) NIH, Bethesda, MD; 3) Hadassah Univ Hosp, Jerusalem, Israel.

Familial Dysautonomia, (FD) is an autosomal recessive neuropathy with a carrier frequency of 1:30 in Ashkenazi Jews. We recently reported that FD is caused by 2 mutations in the I $\kappa$ B kinase complex-associated protein (*IKBKAP*) gene on chromosome 9q31. The 471 kb candidate region for FD contained 8 genes. Prior to the identification of the FD mutations, we screened the coding sequence of each candidate gene and found several SNPs. Using haplotype analysis, 2 of these SNPs enabled us to reduce the candidate region to a 178 kb interval containing 5 genes. We generated the complete genomic sequence of this region in both affected and control cosmids. Direct comparison of the sequences identified 152 differences including 26 variations in the length of dTn tracts, 1 VNTR, and 125 SNPs. Examination of the SNPs in this region reported in dbSNP reveals very little overlap with our polymorphisms. In the 8 coding regions we identified 22 coding sequence SNPs, while dbSNP reports a total of 17. Interestingly, only two of these SNPs are the same. Evaluation of all SNPs (coding and non-coding) across the 178 kb candidate region again reveals little overlap, with dbSNP reporting 83 SNPs while we have identified 125 in the Ashkenazim. Our coding sequence SNPs were identified by sequencing 4 individuals, and the non-coding SNPs were the result of comparing only 2 individuals. Nonetheless, we identified more SNPs during our screen than are reported in dbSNP, despite the fact that they typically result from comparison of a greater number of sequences. Our results demonstrate that the SNP database is not likely representative of specific ethnic isolates, a point that should be considered by investigators using SNPs for linkage and association studies. Further, we demonstrate that screening a very small number of individuals (2-4) in the study population will likely yield a significant number of SNPs in the gene or region of interest.

**Haplotype analysis of chemokine and chemokine receptor polymorphisms.** *V.J. Clark<sup>1,2</sup>, R.J. Peterson<sup>3</sup>, M. Dean<sup>1</sup>.*  
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Chemokines are small intercellular signaling molecules that recruit immune cells to the site of inflammation and infection. Chemokine and chemokine receptor variants influence susceptibility to HIV-1 infection, progression to AIDS, and the efficiency of the immune system. More recently, these proteins have been implicated in tumorigenesis and metastasis in breast and other cancers. Therefore, studies of variation in these genes, and their cell-surface receptors, may be crucial to understanding how gene function affects the complex process of cancer.

Haplotype analysis of Single Nucleotide Polymorphisms (SNPs) has been increasingly important in the study of complex disease. In this study, we have identified haplotypes of SNPs in chemokine and chemokine receptor gene clusters found on chromosomes 3p21, 4q21 and 17q11-12. Forty SNPs in both coding and non-coding regions of chemokine and chemokine receptor genes were genotyped using the 5 nuclease assay (TaqMan). Haplotype frequencies were determined in European-Americans and African-Americans, as well as in native African and Asian populations using the Expectation-Maximization (EM) algorithm. Analyses of pairwise comparisons of SNP frequencies describe the patterns of linkage disequilibrium over tens and hundreds of kilobases in each gene region. To further describe the haplotype structure, pedigree analyses of 40 multi-generation CEPH families have been performed to determine the haplotype phase of whole chromosomes.

These analyses are an important first step in describing the haplotype structure and the structure of linkage disequilibrium in chemokine and chemokine receptor genes. The nature of the extent haplotype structure of these regions will be determined in a study of a worldwide population panel. As a final step, these haplotypes will be used in association studies of case/control samples for breast cancer (n=200), AIDS-related Kaposi sarcoma (n=508), and AIDS-related lymphoma (n=200).

**HLA POLYMORPHISMS IN PAKISTANI ETHNIC GROUPS.** *A. Mohyuddin, Q. Ayub, S. Khaliq, A. Mansoor, K. Mazhar, S. Rehman, R. Qamar, S.Q. Mehdi.* Biomed & Genet Engg Labs, Dr. A. Q. Khan Research Labs, Islamabad, Pakistan.

HLA population genetic analyses have been carried out in many world populations, but no major studies have been performed on the Pakistani populations. In this study the HLA -A, -B, -C, -DRB and DQB1 loci of seven Pakistani ethnic groups have been analyzed in an attempt to study the genetic diversity of Pakistani populations and to examine the phylogenetic relationship of the different Pakistani races with respect to each other as well as to other world populations. The populations selected for the study include Baloch, Brahui, Parsi and Sindhi from the south and Burusho, Kalash and Pathan from the north of Pakistan. HLA typing was carried out using polymerase chain reaction with sequence specific primers (PCR-SSP). Neighbor joining trees based on allele frequencies were constructed and correspondence analysis was carried out. Diversity was observed in the allele and haplotype distribution between the seven Pakistani ethnic groups. Analyses based on HLA- A, -B, -C, -DRB1 and DQB1 allele frequencies revealed the Kalash and Parsi populations to be distinct from each other and the remaining Pakistani populations. The Baloch and Brahui were closely related to one another. The Sindhi were closer to the Pathan and Burusho populations than to the neighboring Baloch and Brahui populations indicating admixture between the northern and southern populations of Pakistan. Phylogenetic and correspondence analyses comparing the Pakistani populations to various other world populations (HLA- A, -B and -C allele frequency data from the 11th and 12th International Histocompatibility Workshops) showed that the Pakistani ethnic groups, with the exception of the Parsi, lie within the cluster of the European and Asian Indian populations. The Parsi population however shows a striking affinity to the Iraqi Jews. These results help in studying the origins of the various Pakistani populations and provide a background for a variety of applications including tissue typing and HLA and disease association studies in Pakistan.



**Genetic diversity in Mongolians and Japanese: A comparison of Y-chromosome, mitochondrial DNA and X-chromosome variations.** *B. Munkhbat*<sup>1,2</sup>, *K. Tounai*<sup>1</sup>, *T. Katoh*<sup>1</sup>, *T. Ikuta*<sup>1</sup>, *H. Ando*<sup>1</sup>, *A. Yamamoto*<sup>1</sup>, *R. Sato*<sup>1</sup>, *G. Oyungerel*<sup>2</sup>, *G. Tamiya*<sup>1</sup>, *N. Munkhtuvshin*<sup>2</sup>, *K. Tokunaga*<sup>3</sup>, *H. Inoko*<sup>1</sup>. 1) Dept. of Molecular Life Science, Tokai University School of Medicine, Isehara, Kanagawa, Japan; 2) National Institute of Health, Mongolian Medical University, Ulaanbaatar, Mongolia; 3) Dept. of Human Genetics, Graduate School of International Health, The University of Tokyo, Tokyo, Japan.

The assessments of genetic diversity in human populations have long been studied by mitochondrial DNA, Y-chromosomal, and various types of autosomal polymorphism. Among various genetic markers used, highly mutable markers such as microsatellites (short tandem repeats, STR) are most suitable for examining recent events and the relationships among closely related populations. Their characteristics such as genome-wide distribution, high degree of polymorphism, ease of the typing by automated multiplex PCR, have resulted in a relatively wide use of microsatellite markers during last decade. The development of highly polymorphic microsatellite markers on the non-recombining region of the human Y-chromosome would allow determining the best haplotypic resolution and therefore would make it the most powerful tool to study genetic diversity in human populations. Therefore, we have attempted to identify novel microsatellite markers on human Y-chromosome. Eighteen novel, Y-specific, and polymorphic microsatellite markers (5 tri-, 10 tetra-, and 3 pentanucleotide) were developed from the comprehensive human sequence data that are accumulating in publicly available databases and applied for population study. Here, we will present the analysis of within- and between-population genetic diversity in 4 Mongolian and Japanese populations using 18 newly identified and 15 known Y-specific markers in comparison with Caucasians and other human populations. Furthermore, the results obtained from the variations in Y-chromosome loci will be compared with the variations in mitochondrial DNA and X-chromosome.

**A Study of the Greek Ancestry of Northern Pakistani Ethnic Groups using 115 Microsatellite Markers. A.**

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Pakistan lies in a region that has witnessed multiple invasions and migrations over the centuries and is therefore composed of diverse ethnic groups. Three northern Pakistani populations, the Pathans, the Burusho and the Kalash claim descent from Greek soldiers who were left in this region after Alexander's invasion of the Indian sub-continent in 327-323 B.C. The Burusho reside in Hunza and Nagar Valleys, which are located in the Karakorum mountains and speak the language isolate Burushaski. The Pathan tribes inhabit the North West Frontier Province of Pakistan and parts of neighboring Afghanistan and speak Pushto, an Indo-European language. The Kalash have been isolated for centuries in the Hindu Kush mountain ranges of northern Pakistan and speak Kalasha, which is also an Indo-European language. To investigate the genetic relationship between extant Greek population and the three Pakistani ethnic groups, forty unrelated samples from each population were analyzed using 115 autosomal microsatellite markers. Tri (17) and tetranucleotide (98) were typed by multiplex PCR and analyzed on ABI 377 sequencer using Genescan software (2.1). VisTA and DISPAN programmes were used for principal component analysis and for calculating the genetic distances between these populations. The heterozygosity values for all loci varied between 0.71 (Kalash) to 0.74 (Greeks). The Kalash population was the least heterozygous and had the lowest number of unique alleles. The principal component analysis of allele frequency data grouped the Pathans and the Burushos with the Greeks. A phylogenetic tree generated using DAS values also separated the Kalash from the other three populations with the bootstrap value of 100% from 10,000 replications. The clustering of the Burusho and Pathans with the Greeks suggests that the gene flow has occurred between these populations.

**Dynamics of microsatellite genetic diversity in the Indian Subcontinent.** *S.S. Mastana*<sup>1</sup>, *G. Sun*<sup>2</sup>, *S.S. Papiha*<sup>3</sup>, *R. Deka*<sup>2</sup>. 1) Dept Human Sci, Human Gen Lab, Loughborough Univ, Loughborough, England; 2) Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267. USA; 3) Department of Human Genetics, University of Newcastle on Tyne. England.

We have analyzed genetic variation at 13 STR loci (CODIS core loci) in a sample of 16 ethnically and geographically diverse endogamous caste and tribal populations of the Indian subcontinent. A wide spectrum of allelic distribution at different loci was visible 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. FGA locus was the most polymorphic in a majority of populations with 17 observed alleles. Other highly polymorphic loci in Indian subcontinent include D21S11(15 alleles) and D8S1179(10 alleles). 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. Comparisons are in progress with other genetic markers including mtDNA and Y-chromosome. We also computed forensic and paternity statistics. Lowest match probability and highest exclusion probability was observed for the FGA locus. Combined match probability was low ( $1$  in  $8.99 \times 10^{12}$  to  $2.9 \times 10^{13}$ ), and combined exclusion probability was  $> 99.999\%$ . There was no evidence of association of alleles 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 study was supported by NIH grant GM45861, NIJ grant 98-LB-VX-002 and funds from Loughborough University.

**A nuclear marker for the southern route out of Africa.** A.D. Lovell<sup>1</sup>, E. Zietkiewicz<sup>1</sup>, V. Yotova<sup>1</sup>, M. Batzer<sup>3</sup>, D. Labuda<sup>1, 2</sup>. 1) Research Center, Sainte Justine Hospital, and; 2) Pediatrics Dept, U of Montreal, Montreal, QC, Canada; 3) LSU, Baton Rouge, LA, USA.

The out of Africa scenario proposes two major routes of modern human dispersal from Africa. The northern route, through the Levant into Eurasia, is supported by fossil remains and genetic data; an earlier, southern route, through the horn of Africa and along the southern Asian coast to South East Asia, awaits experimental documentation. We present here a nuclear marker that provides support for the southern route out of Africa. The *dys44* haplotype consists of 35 segregating sites within an 8kb segment flanking exon 44 of the dystrophin gene on Xp21. *Dys44* haplotypes were analyzed in a sample of 1315 chromosomes from all over the world. The haplotype family B002 is found at high frequencies in Sub-Saharan Africa (26%), East Asia (30%) and Oceania (49%), and at very low levels in Europe (4%) and the Americas (1%). Comparing populations from the putative southern and northern migrations statistically supports ( $p < 0.001$ ) the observation that B002 follows the southern route. Four alleles of a T-repeat, occurring within the *dys44* segment, are associated with B002 haplotypes in Africa, while only one of these alleles is found outside Africa, suggesting that B002 did not originate in Asia. Furthermore, the frequency of an *Alu* insertion, within the dystrophin intron 44, increases dramatically from Africa (9%) to Melanesia (60%); this pattern is consistent with a lack of substantial back-flow of genes from Asia to Africa. Our findings suggest that B002 distribution provides a genetic record of the hypothesized southern route out of Africa. To add resolution and further test B002 as a marker, samples from Eastern Africa and Southern Asia need to be analyzed. (*Supported by Canadian Institutes of Health Research*).

**Variation in PPARb is associated with lower fasting glucose and insulin levels in Hispanic and Non-Hispanic white individuals.** *S.P. Moffett<sup>1</sup>, M. Barmada<sup>1</sup>, E. Feingold<sup>1</sup>, C.M. Damcott<sup>1</sup>, B. Guner<sup>1</sup>, J.A. Marshall<sup>2</sup>, R.H. Hamman<sup>2</sup>, J.M. Norris<sup>2</sup>, R.E. Ferrell<sup>1</sup>.* 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) University of Colorado, School of Medicine, Denver, CO.

The peroxisome proliferator activated receptors (PPARs), members of the nuclear receptor subfamily, influence the expression of genes involved in pathways such as fatty acid metabolism, glucose transport and energy regulation. Insulin can activate the PPARs by phosphorylating the protein. PPARb is one of the three PPAR isoforms and is expressed at moderate levels in most tissues, although high levels are found in the large intestine. We screened the coding sequence of the PPARb gene for common polymorphisms to determine if genetic variation in the gene is associated with glucose or insulin levels. A silent C to T substitution was found in exon 6 at nucleotide 72054 (Genbank accession # AL022721) using SSCP and sequencing for mutation detection. This variant, PPARb6, alters an Nde I restriction site which was used to genotype the polymorphism in a group of Hispanic (H) and Non-Hispanic (NH) white individuals enrolled in the San Luis Valley Diabetes Study (n=764). Allele frequencies in the two ethnic groups were similar ( $q=0.137$  in H and  $q=0.127$  in NH). Analysis of variance showed associations between the less common allele of PPARb6 and lower fasting glucose ( $p=0.013$ ), fasting insulin ( $p=0.034$ ) and HOMA IR ( $p=0.013$ ), a derived measure of insulin resistance. Regression models including PPARb6, sex and skin reflectance as a measure of ethnicity were then tested. PPARb6 and skin reflectance were found to be significant predictors of variation in  $\ln(\text{fasting insulin})$  ( $p=0.001$ ) and  $\ln(\text{HOMA IR})$  ( $p=0.0003$ ) while PPARb6, skin reflectance and sex were all significant predictors of  $\ln(\text{fasting glucose})$  ( $p<0.0001$ ). These results suggest a role for PPARb in glucose regulation and insulin metabolism independent of sex and ethnicity in this population.

**Genomic approaches to DNA identification of kidnapped children: Progress of the Argentina project.** *K.N.*

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During the Argentine military dictatorship of 1975-1983 at least 250 young children were taken from their biological families. Ongoing efforts by the Abuelas de Plaza de Mayo to identify these missing children, now young adults, have integrated DNA evidence. The challenge is matching rediscovered children with their respective biological families using incomplete information. We have found mtDNA sequences to be particularly useful. Any mitochondrial relative will match a missing child, a particular advantage given that both parents are deceased in the majority of these families. For each case, we sequenced 675 bp at the mtDNA origin of replication and maintain a database of mtDNA sequences of surviving relatives, discovered children of unknown identity, and population controls. Diversity of sequences in this population is high; 81% of families have mtDNA sequences unique in the Argentine database. Unique sequences reflect the occurrence of rare variants on ancestral haplotypes. The average difference between sequences from unrelated persons in the database is 11.2 nucleotides. If the mtDNA sequences of a rediscovered child match a surviving family, we genotype multiple nuclear loci to test the hypothesis of relationship. It is useful to evaluate allele-sharing at multiple loci of all available relatives with an undisputed sibling of the disappeared child. This proportion of shared alleles is compared to the proportion shared by the child of unknown identity with the same relatives. This approach provides an alternative to methods that depend on population-specific allele frequencies.

**Global variation in the IL-10 C-592A and eotaxin C-1382A AIDS-resistance SNPs.** *J.J. Martinson, P. Aldred.*  
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Many host loci are known to affect the rate of progression to AIDS following infection with HIV-1. In the early stage of infection, HIV-1 targets macrophages: the cytokine IL-10 inhibits macrophage development, reducing the available population of target cells for HIV, but a C/A SNP in the IL-10 promoter affects rates of HIV infection and AIDS progression. The A form of the SNP increases these, probably by down-regulating IL-10 and thereby enhancing macrophage proliferation. The chemokine eotaxin is one of the normal ligands for CCR3, a chemokine with a minor role in HIV infection. A C/A SNP in the eotaxin promoter nevertheless affects the rate of HIV infection, but not of AIDS progression in individuals who do become infected. The A allele is protective, but the mode of action whereby eotaxin achieves this is presently not known. We have determined the global distribution of these two SNPs using a large sample of DNAs typed previously for SNPs in CCR5. Each of these SNPs is polymorphic in all populations studied, although the allele frequencies vary greatly. For the IL-10 accelerator A allele, the highest frequency of 65% was seen in southern Chinese. Other Asian populations showed this allele at frequencies between 15% and 40%. In Caucasian populations, the A allele frequency varied from 12-25%, whereas in African populations this allele was slightly more common (30-35%). For eotaxin, Caucasian populations generally showed a slightly higher frequency of the protective A allele (15-40%) than Asians (5-20%) or Africans (2-7%). African populations had both low levels of the eotaxin resistance allele and high levels of the IL-10 susceptibility allele, which may partly explain the rapid spread of AIDS in sub-Saharan Africa. Data from these two loci alone show that there is a great deal of variation between populations in the levels of innate resistance and susceptibility to HIV infection and AIDS progression. As more loci are found to affect these processes, inter-population differences in disease resistance will become ever more important in the prediction of viral transmission rates and in the development of AIDS vaccines.

**Patrilinear origins of Pakistani ethnic groups.** *R. Qamar*<sup>1,2</sup>, *Q. Ayub*<sup>1,2</sup>, *A. Mohyuddin*<sup>1,2</sup>, *K. Mazhar*<sup>1</sup>, *A. Mansoor*<sup>1</sup>, *S. Khaliq*<sup>1</sup>, *T. Zerjal*<sup>2</sup>, *C. Tyler-Smith*<sup>2</sup>, *S.Q. Mehdi*<sup>1</sup>. 1) Biomedical and Genetic Engineering Division, Dr. A. Q. Khan Research Laboratories, Islamabad, Pakistan; 2) Department of Biochemistry, University of Oxford, Oxford, UK.

Pakistan lies on the postulated route followed by anatomically modern *H. sapiens* out of Africa. Besides being the cradle of the Indus Valley Civilization this region of Asia has been influenced by various human migrations and invasions over the millennia. The origins of present day Pakistani populations was investigated by typing 16 biallelic unique event polymorphisms, and 21 multiallelic, short-tandem-repeat (STR) loci, including six previously unreported STRs, mapping on the non-recombining portion of the human Y chromosome. More than 800 individuals representing 15 Pakistani ethnic groups were analyzed. The northern Pakistani ethnic groups included in this study were the Balti, Burusho, Hazara, Kalash, Kashmiri, Meos, Pathans and Rajputs. The southern groups included the Baloch, Brahui, Makrani Baloch, Makrani Negroid, Mohannas, Parsi and Sindhis. The combination of the biallelic markers identified 11 stable Y chromosomal lineages, referred to as haplogroups, in the Pakistani population. Notable differences were observed in the Hazara and Mohanna ethnic groups. The former claim to be descendants of Genghis Khan and this fact is substantiated by analysis of their haplogroup 10 Y chromosomes, which is found at high frequencies in populations from Central Asia, China and Mongolia. The Mohannas are reputed to be the indigenous populations of Sindh in southwestern Pakistan. Their Y chromosomal lineages are significantly different from the other Indo-European speaking ethnic groups residing in Pakistan, including their neighboring Sindhi populations. The language isolate group of Burushos and the Dravidian speaking Brahuīs were not much different from their geographic neighbors within Pakistan. Based upon the principal component analysis of haplogroup frequencies the Pakistani ethnic groups were closely related to each other and the Europeans. Most Pakistani populations had the M20 A to G transition. This polymorphism has not been found at a significant frequency elsewhere in the world.



**The relationship of Apolipoprotein E and ACE to coronary heart disease among Southwest Hispanics, African Americans and Caucasians.** *D.P. Rojas<sup>1</sup>, L.H. Mateus Pereira<sup>1, 2</sup>, I. Fernandez<sup>1</sup>, M.C. Terreros<sup>1, 4</sup>, A. Socorro<sup>1</sup>, R.J. Herrera<sup>1</sup>, F. Huffman<sup>3</sup>.* 1) Biological Sciences, Florida International University, Miami, FL; 2) Depto. Genetica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; 3) Department of Dietetics and Nutrition, Florida International University, USA; 4) Comision de Investigaciones Cientificas (CONICET), Argentina.

Coronary heart disease (CHD), a leading cause of mortality and morbidity in industrialized countries is a multifactorial disease caused by the interaction of polymorphic inheritance and environmental factors. An understanding of the genetic make up that predisposes and contributes to development of risk factors for CHD is critical to understand the pathogenesis of this disease. The objective of this study is to determine the relationship of the ACE (angiotensin converting enzyme) Alu element insertion polymorphism and Apo E polymorphism (E2, E3, and E4 variants) as factors that contribute to the development of CHD. The human DNA samples used in this work are from three different ethnic backgrounds Hispanic, African American of non-Hispanic origin, and White of non-Hispanic origin with equal number of men and women at each group. There is no previously reported information on young diverse populations (college students) that focuses on ACE and Apo E polymorphisms. The DNA was extracted from serum using a Quiagen mini kit. The Alu insertion polymorphism used in this study was ascertained by PCR-based method using gene counting to obtain the insertion and lack of insertion frequencies. For the Apo E gene we are using a RFLP method described elsewhere (REF). The results of this study may help to identify the factors involved in CHD among young sub-population groups of different ethnicities. Thus it can provide greater insight into the potential utility of admixed populations for the mapping of complex traits.

**Diversity of paternal lineages in populations of Siberia and Central Asia.** *V.A. Stepanov<sup>1</sup>, V.P. Puzyrev<sup>1</sup>, V.N. Kharkov<sup>1</sup>, S. Roots<sup>2</sup>, M.G. Spiridonova<sup>1</sup>, Z.O. Soltobaeva<sup>3</sup>, R. Villem<sup>2</sup>.* 1) Lab Evolutionary Genetics, Inst Medical Genetics, Tomsk, Russia; 2) Estonian Biocentre, Tartu, Estonia; 3) Kirghiz Medical Academy, Bishkek, Kirghizia.

Y-chromosome variation was analyzed in a sample of 600 individuals from nine indigenous populations of Siberia (Tuva, Buriat, Northern and Southern Altai, Evenk, Yakut) and Central Asia (Kirghiz, Uzbek, Tajik). Paternal lineages was constructed using 11 biallelic and 7 microsatellite loci. Distribution of 10 biallelic haplogroups (HGs) forming the parsimonious genealogical tree displays distinct ethno-geographic differences. Most frequent haplogroups in the total sample were HG3 (26.4%) and HG16 (23.2%). HG16 encompassed 35-90% of Y chromosomes in Yakuts, Buriats and Evenks, whereas HG3 reached the highest frequency in Altai and Kirghiz populations. Principal component analysis of biallelic data shows that populations of Central Asia and Altay-Sayan region clearly separated from the Eastern Siberians because of high frequency of HG16 in the latter. Using the molecular variance of microsatellite loci within the biallelic HGs we estimate the time to the most recent common ancestor for several frequent HGs. According our data, the coalescent age of HG3 which is widely distributed in Europe and Northern Asia is around 13200 YBP. The STR diversity associated with HG12 provided age estimate of about 6250 YPB, and the age of HG16 which is derivate of HG12 was estimated as about 4250 YPB. Our findings suggest that the Y-chromosome variation correlates with antropological characteristics of the populations and their spatial localization rather than their linguistic affiliation.

**East European gene geography of 3'ApoB VNTR polymorphism.** *D.A. Verbenko<sup>1</sup>, O.P. Balanovsky<sup>1</sup>, E.V. Balanovskaya<sup>2</sup>, E.K. Khusnutdinova<sup>3</sup>, S.A. Limborska<sup>1</sup>.* 1) Human Genetics Department, Institute of Molecular Genetics of Russian Academy of Sciences, Moscow, Russian Federation; 2) Research Centre for Medical Genetics of Russian Academy of Medical Sciences, Moscow, Russian Federation; 3) Institute of Biochemistry and Genetics (Ufa Scientific Centre) of Russian Academy of Sciences, Ufa, Russian Federation.

Apolipoprotein B gene VNTR (variable number of tandem repeats) polymorphism is commonly used to distinguish individuals in forensic science. Moreover, ApoB minisatellite diversity combined with other ApoB gene polymorphisms is one of the major parts in atherosclerosis association studies. Last time papers shown the great possibilities of use the minisatellite in microevolutionary processes research. In spite on worldwide study of 3'ApoB distribution East European populations are not generally presented yet. Our data are based on healthy individuals polymorphisms from East Slav (Russians, Byelorussians, Ukrainians), Volgo-Uralic (Bashkirs, Komis, Chuvashes, Tatars, Udmurts, Mordvinians, Mari), Adhyg and Kalmyk populations.

The principal components of variability were extracted from all 3'ApoB VNTR allele frequencies to reveal integrative diversity. The complex estimate of the prediction probability of all points of the real was taken into account in order to construct principal component variability maps properly. A set of geographic variability maps was constructed on the base of principal component digital matrixs for all map points.

The map of first principal component of variability reflect homogenous plateau encompassed with two variability gradients. The plateau is situated on Russian Plain - which is the place of East Slavs living. One of the gradients spread from Kalmykia through Karpaty Mountains to Baltic Sea. Another gradient has its maximum in Ural Mountains. Both gradients have common minimum in Kalmykia. The minimum position may be explained with Oriental lineage of Kalmyks. One can see that ApoB geographic distribution in East Europeans reflects ethnic and geographis borders of the region.

**Single nucleotide polymorphisms in 539 human genes.** *J.A. Schneider, T. Acharya, R. Jiang, G.F. Vovis, J.C. Stephens.* Population Genomics, Genaissance Pharmaceuticals, New Haven, CT.

We discovered 6,808 SNPs in approximately 1.4 Mb of genomic DNA from 539 human genes. DNA sequencing was performed on one chimpanzee and 82 unrelated individuals including African-Americans, Asians, European-Americans and Hispanic Latinos. The data were collected from specific gene regions including exons, exon-intron boundaries, untranslated regions and 5' and 3' flanking regions. Characterization of these SNPs revealed information about mutational, selective and demographic processes that have influenced patterns of human genetic diversity. We found that ~40% of our SNPs were mutations of either base in a CpG dinucleotide, consistent with the fact that CpG dinucleotides are prone to methylation. While numerous SNPs were population-specific, approximately 20% were observed in all four ethnic groups. For some SNPs, we found that the rare human allele matched the corresponding chimpanzee nucleotide, contrary to the expectation that the common human allele should be ancestral. Recurrent mutation or recent selection could explain this observation. The distribution of polymorphisms within these genes may indicate a historical population expansion, since 481 out of 539 genes had a negative Tajima's D value. Together, these results suggest that human genetic diversity is the result of a complex interplay of evolutionary forces.

**HUMAN X-CHROMOSOMAL LINEAGES IN EUROPE REVEAL MIDDLE EASTERN AND ASIATIC**

**CONTACTS.** *F.X. Xiao*<sup>1</sup>, *E. Zietkiewicz*<sup>1</sup>, *V. Yotova*<sup>1</sup>, *J.P. Moisan*<sup>3</sup>, *I. Arrieta*<sup>4</sup>, *D. Labuda*<sup>1, 2</sup>. 1) Research Center, Hospital Sainte-Justine, and; 2) Pediatrics Dept, U of Montreal, Montreal, QC, Canada; 3) CHRU, Nantes, France; 4) UPV, Bilbao, Spain.

European genetic data using classical genetic markers and DNA polymorphisms revealed an east-west frequency gradient often interpreted as evidence for the migration of Neolithic farmers spreading agriculture from the Middle East. However, it is not clear how to discern the genetic record tracing routes of the first peopling of Europe during the Upper Paleolithic or of other, later movements. We have analyzed nuclear haplotypes composed of 35 polymorphic sites in an 8 kb intronic DNA flanking exon 44 of the human dystrophin gene on Xp21 in a worldwide sample of chromosomes. Out of ten haplotype families identified, three are restricted to Africa and seven are shared by populations from all the continents. Within Eurasia, haplotype B001 is found at similar, high frequencies in Europe, the Middle East and Asia, while haplotype B003 is mainly concentrated in Europe and the Middle East. Haplotype B002 decreases in frequency from the Middle East to Europe, while haplotypes B005, B006 and B008 show the opposite tendency. On the other hand, we also observe frequency gradients from Asia to Europe: decreasing in the case of haplotypes B002 and B005, and increasing in the case of B003. The distribution of B006, a strong marker of Northern populations, appears relatively even from Europe to Central Asia. These patterns give a signal of the gene flow being as strong between the Middle East and Europe as between Central Asia and Europe. (*Supported by CIHR; F.X.X. has a fellowship of HSJ Research Center*).

**Yq microdeletion screening in 120 Iranian infertile men.** A. Hatami<sup>1</sup>, M.A. Sadighi Gilani<sup>2</sup>, L. Abbasi Moheb<sup>1</sup>, P. Afsharian<sup>3</sup>, S. Molla Mohammadi<sup>2</sup>, M.K. Javan<sup>1</sup>, H. Pak<sup>2</sup>, H. Najmabadi<sup>1</sup>. 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Andrology department, Rooyan institute, Tehran, Iran; 3) Department of Clinical and Experimental Genetics, Rooyan Institute.

Infertility affects 10-15% of couples seeking to have children. Male factor is the leading cause in about 50% of cases. Recent studies have established the concept of a genetic basis for male infertility. Although macroscopic deletions are uncommon in infertile men (0.5%), the existence of microdeletions in AZF(Yq 11.22-23) has been reported in 3-20% of idiopathic oligo/azoospermic men. DAZ and RBM are two multicopy gene families which are expressed only in testes and have an important role in spermatogenesis. They are located at AZFc and AZFb (interval 6 of Yq) respectively. Microdeletions in these regions cause severe oligozoospermia or azoospermia. In IRAN, there has been no comprehensive study on microdeletion screening of infertile men. In this study 23 STS were selected to screen for Yq microdeletions. One hundred and twenty azoospermic and oligozoospermic individuals were selected. Cytogenetic, hormonal and other causes of infertility have been ruled out in these men. Our results indicate 8 out of 120 individuals with Yq microdeletions (6.6%).

**Sample size calculations for association studies of gene-gene interaction.** *W.J. Gauderman.* Dept Preventive Medicine, Univ Southern California, Los Angeles, CA.

In the study of complex diseases, it is important to test hypotheses related to gene-gene (GxG) interaction. The success of such studies depends critically on obtaining adequate sample sizes. In this paper, we describe a method for computing sample size requirements for studies of GxG interaction. We focus on three case-control study designs, including the matched case-control, case-sibling, and case-parent designs. All three types of studies can be analyzed using conditional logistic regression, which is used as a unifying theme in the development of sample size calculation methods. Comparisons of sample size requirements for detecting GxG interaction indicate that the case-parent design typically requires half the number of matched sets compared to the other two designs. As an example, we describe a study of two common recessive genes for which 270 matched case-control pairs would be required to detect a GxG interaction of moderate magnitude with 80% power. By comparison, the same study would require 319 case-sibling pairs, but only 146 case-parent triads. A software program that computes sample size for studies of GxG, and for studies of gene-environment (GxE) interaction, is freely available and may be downloaded from the website <http://hydra.usc.edu/gxe>.

**Identifying tuberculosis disease resistance genes using Quantitative Phenotype Analysis.** A.A. Awomoyi<sup>1</sup>, A. Marchant<sup>2</sup>, G. Sirugo<sup>3</sup>, K. McAdam<sup>3</sup>, S.A. Tishkoff<sup>1</sup>, J. Blackwell<sup>4</sup>, M. Newport<sup>4</sup>. 1) Department of Biology, University of Maryland College Park, MD.20742; 2) Institute of Molecular Medicine, Oxford UK; 3) Medical Research Council Labs, Fajara The Gambia; 4) Cambridge Institute for Medical Research Cambridge UK.

Tuberculosis (TB) is a complex trait in which host genetics, environmental factors and the pathogen play a role in development of the disease phenotype, making it difficult to identify specific genes involved. Gene mapping may be simplified by regarding the disease phenotype as a constellation of underlying quantitative traits which can be studied independently. Macrophage activation, a crucial stage in initial host defense against intracellular pathogens, is a complex process which involves several genes: defects of macrophage activation may result in increased susceptibility to TB. We used in vitro cytokine responses to lipopolysaccharide (LPS), and lipoarabinomannan (LAM), in healthy blood donors as phenotypic markers of macrophage activation in conjunction with candidate gene association studies to answer two questions relating to genetic regulation of macrophage activation in TB: do macrophage candidate genes regulate microbial induced responses and do macrophage candidate genes influence susceptibility to TB? TNF, IL-1 and IL-10 responses showed considerable heterogeneity in this population. Polymorphisms in macrophage candidate genes were analysed in relation to this phenotypic variation. These included new polymorphisms detected within the promoter of the IFNGR1 gene that occur within or close to functional elements. The TNFA -863, LTA NcoI, IL-1RN and NRAMP1 (INT4) alleles were shown to influence macrophage cytokine levels significantly. A case control association study conducted in parallel identified association between the following alleles and TB: TNFA -308, TNFA -238, IL1B, IFNGR1, IL9, NRAMP1 (INT4) and NRAMP1 CA repeat. These results indicate that analysis of quantitative traits underlying a complex disease trait may be a useful tool in mapping candidate genes in infectious diseases such as TB.



**Association between the apolipoprotein E polymorphism and the severity of coronary artery disease in the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) Study.** *Q. Chen*<sup>1</sup>, *S.E. Reise*<sup>2</sup>, *D. Pauly*<sup>3</sup>, *B. Sharaf*<sup>4</sup>, *D. McNamara*<sup>2</sup>, *R. Holubkov*<sup>5</sup>, *C.N. Bairey Merz*<sup>6</sup>, *M.I. Kamboh*<sup>1</sup>. 1) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Department of Medicine, University of Pittsburgh, Pittsburgh, PA; 3) Department of Medicine, University of Florida, Gainesville, FL; 4) Department of Medicine, Rhode Island Hospital, Providence, RI; 5) Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA; 6) Department of Medicine, Cedars-Sinai Research Institute, Pittsburgh, PA.

Genetic variation in the apolipoprotein E (APOE) gene is a significant determinant of variation in plasma cholesterol levels and it also affects the risk of coronary artery disease (CAD). In this study, we examined the association of the APOE polymorphism with coronary stenosis in 575 white women from the NHLBI-sponsored Women's Ischemia Syndrome Evaluation (WISE) study, a multicentered study designed to address issues related to ischemic heart disease diagnosis and pathophysiology in women. Subjects were classified as having normal (<20% stenosis, n=224), mild/minimal disease (20 to 49% stenosis, n=148) and significant disease (<sup>≥</sup>50% stenosis, n=203). Logistic regression was used to compute adjusted odds ratios (ORs) and 95% confidence intervals (CI). The frequency of the *APOE*\*4 carriers (E2/E4, E3/E4, E4/E4) was significantly higher in the diseased group (<sup>≥</sup>20% stenosis) compared to the normal group (<20% stenosis) with an OR of 2.04 (95% CI: 1.29-3.23; p=0.0023). Similarly, the stratification of the data based upon the number of diseased vessels (none, single, double and triple) showed that the frequency of *APOE*\*4 carriers was significantly higher in the triple diseased vessel group than the combined group of none, single and double diseased vessels (OR=4.36, 95% CI=2.09-9.10; p<0.0001). These data support the hypothesis that the *APOE*\*4 allele is a risk factor not only for the presence of CAD, but also for the angiographic severity of the disease in white women.

**Interaction between the Hepatic Lipase 514(C/T) Polymorphism and Dietary Fat Intake Modulates HDL-C Levels and HDL Subfractions: The Framingham Heart Study.** *L.A. Cupples<sup>1</sup>, K. Tucker<sup>2</sup>, D. Corella<sup>2</sup>, S. Demissie<sup>3</sup>, P. Couture<sup>2</sup>, E.S. Tai<sup>2</sup>, P.W.F. Wilson<sup>4</sup>, E.J. Schaefer<sup>2</sup>, J.M. Ordovas<sup>2</sup>.* 1) Dept Epid/Biostatistics, Boston Univ Sch Public Health, Boston, MA; 2) Lipid Metabolism Laboratory, Jean Mayer-USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA; 3) Data Coordinating Center, Boston Univ Sch Public Health, Boston, MA; 4) Boston Univ School of Medicine, Boston, MA.

Hepatic lipase (HL) is a key determinant of HDL metabolism that catalyses the hydrolysis of triglycerides and phospholipids in lipoproteins. Four polymorphisms in complete linkage disequilibrium have been identified in the region upstream of the transcription initiation site of the HL gene (LIPC), defining what is known as the -514T allele. The -514TT genotype is associated with decreased HL activity and increased HDL-C levels. However, the effect is variable among populations, suggesting gene-environment interactions. We have examined interaction effects between the 514(C/T) LIPC polymorphism, dietary fat and HDL-related measures in a sample of 1314 males and 1353 females participating in a large prospective study: The Framingham Offspring study. Our data shows that carriers of the TT genotype and consuming 15% dietary fat have HDL-C concentrations 10 mg/dL higher than TT carriers consuming 35% of energy as fat. Conversely, carriers of the CC genotype consuming a low fat diet have lower HDL-C levels (-8 mg/dl) as compared with those consuming a high fat diet. A similar interaction was observed for large HDL (HDL2) levels, whereas the opposite effect was seen for small HDL (HDL3) levels. Our data, in this large, population-based cohort, demonstrate a significant interaction between the 514(C/T) LIPC polymorphism and dietary fat that modulates HDL-C levels and HDL subclasses in this population. Therefore, the LIPC locus is a significant determinant of the marked individual variability in plasma HDL-cholesterol responses to fat intake. Specifically, TT subjects have an impaired adaptation to higher fat diets that could result in higher cardiovascular risk.

**Genome-wide scan for loci affecting normal adult height in the Framingham Study.** *N. Mukhopadhyay<sup>1</sup>, D.N. Finegold<sup>1</sup>, M. Larson<sup>2</sup>, L.A. Cupples<sup>3</sup>, R.H. Myers<sup>4</sup>, R. Ferrell<sup>1</sup>, D.E. Weeks<sup>1</sup>.* 1) Dept Human Genetics, Univ Pittsburgh/Sch Pub Health, Pittsburgh, PA; 2) Framingham Heart Study, Framingham, MA; 3) Boston University/ School of Public Health, Boston, MA; 4) Boston University/ School of Medicine, Boston, MA.

Height is an ideal example of a polygenic quantitative trait shared by all humans. Previous linkage analysis studies have focused on growth-related factors and individuals with abnormally short stature. Since findings from plants and animals suggest that often a large proportion of the variance for a polygenic trait is controlled by a small number of genes with significant effects, we analyzed data from 1,720 individuals from 333 families from the Framingham Heart Study in order to map loci influencing normal adult height. These individuals are from two cohorts, the first ascertained in 1948 via a random sample of households in Framingham, MA, and the second Offspring cohort in 1971. Individuals in the original cohort have undergone biennial examinations since inception of the study, while individuals in the Offspring cohort have been examined usually every 4 years. The first height measurement for individuals between the ages 20-55 years were analyzed using variance component linkage analysis on extended pedigree structures and extended Haseman-Elston linkage analysis on the component nuclear families with sex and cohort as covariates. Marker data were generated by the Marshfield Mammalian Genotyping Service for 401 markers on chromosomes 1-22, 24 markers on X, and 2 markers in the pseudo-autosomal region of the X chromosome. Nine chromosomes with 11 peaks with  $-\log P$ -values greater than 2.0 were detected. Three chromosomes demonstrated peaks with  $-\log P$ -value greater than 3.0. None of the regions with significant  $-\log P$ -values corresponded to the location of known genes in the neuro-endocrine growth hormone axis. We are currently evaluating (in silico) the regions under these peaks for candidate genes contributing to final adult height. D.N. Finegold and D.E. Weeks contributed equally to this work. The data are from the National Heart, Lung and Blood Institute's Framingham Heart Study at Boston University.

**Testing the association of quantitative traits and haplotypes considering treatment effects.** *Z. Meng<sup>1,2</sup>, D.V. Zaykin<sup>1</sup>, M.G. Ehm<sup>1</sup>, B.S. Weir<sup>2</sup>*. 1) Population Genetics, GlaxoSmithKline Inc, Res Triang Prk, NC; 2) Bioinformatics Research Center, Department of Statistics, North Carolina State University, Raleigh, NC.

To study how genes are related to efficacy and safety of a medicine, pharmaceutical companies are genotyping multiple markers in candidate genes in samples collected in clinical trials. Challenges for analysis include lack of validated statistical genetic methods, multiple correlated genetic markers, ethnic diversity, and low sample sizes. The development of methods of analysis that take into account the nature of genetic data including allelic, genotypic, and haplotype effects in addition to ethnic diversity are critical to extracting maximum information. We propose a model relating a single marker to a quantitative trait by separating the allelic additive and dominance effects, and have extended the results to haplotypes. We also consider the effects of treatments and their interactions with markers on the quantitative trait.

We illustrate the performance of our method on simulated and real data. We compare our model to an analysis of variance approach based on marker genotypic classes. We illustrate the power of utilizing multiple markers with a haplotype approach versus single markers.

**Determination of genotypic marker information to optimally evaluate the role of candidate genes.** *A.S. Jannot<sup>1,2</sup>, M. Reese<sup>2</sup>, C. Krähenbühl<sup>2</sup>, B. Cutayar<sup>2</sup>, F. Clerget-Darpoux<sup>1</sup>.* 1) Unité 535, INSERM, Le Kremlin-Bicêtre, France; 2) ValiGen, La Défense, France.

In multifactorial diseases a strategy is to focus on candidate genes to evaluate their effect on the disease, in other terms to model the correlation between genotypes and phenotypes. The MASC (Marker-Association-Segregation-Chi 2) method (Clerget-Darpoux et al, 1988) was designed for such modeling. Because of the high number of markers (usually SNPs) available on each candidate gene, one has to deal with a very high number of possible genotypes. One problem then is to decide which categories of genotypic markers to use for efficient modeling. The aim of this study is to find the minimum number of genotypic categories offering the best discrimination between groups of genotypes that have a similar distribution of their phenotypes.

We compare the power of several discrimination methods based on the analysis of variance, considering different strategies, i.e. studying the genotypes of each SNPs separately or as the whole set of SNPs. We perform our study - using 6 SNPs in the *CETP* (cholesterol acetyl transferase) gene - with different underlying genetic models involving one or two loci in a quantitative trait assuming different types of interaction between the loci.

We show that, under interaction models with a low marginal effect of each SNP, the methods, which study the effect of each SNP separately, do not offer a good discrimination of the genotypes. In such cases, the use of the complete set of SNPs may be much better in detecting the right categories for the discrimination of the genotypes.

In most current studies, the first step to reduce the number of SNPs is to apply ANOVA on each SNP separately. We show that by doing so, some genetic effects in complex disease may not be detected and that some candidate genes may be wrongly discarded in this early step.

**Association of 5' untranslated region deletion/insertion polymorphism in the ATP-binding cassette transporter A1 (ABCA1) gene with plasma lipid levels in Asian populations.** *J.H.H. Tan<sup>1</sup>, C.K. Heng<sup>1</sup>, H.Y. Yang<sup>2</sup>, Y.S. Tan<sup>3</sup>, M.C. Tong<sup>3</sup>, P.S. Low<sup>1</sup>, N. Saha<sup>1</sup>.* 1) Department of Pediatrics, National University of Singapore; 2) Department of Biochemistry, National University of Singapore; 3) National Heart Center, Singapore.

The ATP-binding cassette transporter A1 (ABCA1) plays a key role in cholesterol and phospholipid efflux. Mutations in the gene are related with depressed high density lipoprotein-cholesterol (HDL-C) levels and increased incidence of coronary artery disease (CAD) among Tangier disease patients. In the general population, polymorphisms in the gene may also contribute to subtle variations in lipids/lipoproteins. We study a previously observed 1 nucleotide deletion/insertion (2G, 3G alleles respectively) polymorphism in the 3 main ethnic groups of the Singapore male population. It occurs in the 5' untranslated region of exon 2. In the presence of the 3G allele, a *Bst*I restriction site is created. 3G allele frequencies between healthy and CAD groups in each race were similar (healthy: Chinese=0.16, n=247; Malays=0.19, n=176; Indians=0.27, n=231; vs CAD: Chinese=0.16, n=515; Malays=0.20, n=113; Indians=0.27, n=160). Within each of the 3 healthy cohorts, we tested for effect of the polymorphism on lipid profiles. Among Malays, individuals with 3G3G genotype had lower apolipoprotein A1 level ( $p=0.046$ ). Indians with the 3G3G genotype showed higher total cholesterol (TC) ( $p=0.014$ ) and low density lipoprotein-cholesterol (LDL-C) ( $p=0.025$ ); they also tended to have lower HDL-C levels ( $p=0.067$ ). In this population study, we demonstrate that less favorable lipid/lipoprotein profiles in healthy male individuals are associated with certain 2G/3G genotypes and its effect is ethnicity-dependent.

**Introduction of the multivariate Dale model in genetic association studies.** *K. Van Steen<sup>1</sup>, N. Tahri<sup>2,3</sup>, G.*

*Molenberghs<sup>1</sup>.* 1) Center for Statistics, Limburgs Universitair Centrum, Diepenbeek, Belgium; 2) INSERM U.525, C.H.U. Pitié Salpêtrière, Paris, France; 3) Genset SA, Genomics Research Center, RN7 site snecma, Evry, France.

Large-scale case-control association studies based on a dense map of markers have received increasing attention in recent years. A possible explanation for this growth is their potential use in identifying genomic regions of disease predisposing mutations. It is well known that most regions of relevance to a disease may have several base positions within them that when mutated either directly contribute to disease susceptibility or have some related physiopathological effects.

Until now, one of the most common methods to study the combined effects of several markers at different positions is logistic regression. For this approach we observe that if genotypic marker information is incorporated in a classical logistic model at the covariate level, due to the potential linkage disequilibrium between the markers, we have to face the problem of multicollinearity (Neter et al., 1996). Hence, sensitive statistical methods are needed in the identification of multiple susceptibility genes associated to complex diseases.

Here we introduce the multivariate Dale model (Molenberghs and Lesaffre, 1994), which is sometimes referred to as the multivariate (global) odds ratio model, into the world of statistical genetics. Not only is this model more intuitive, the use of a multivariate model also enables the investigation of existing correlations between markers. Interpretations remain straightforward and similar to those drawn in a classical logistic modeling framework. Benefits and drawbacks of the multivariate Dale model are pointed out. While applying the model to real-life case-control samples genotyped on SNPs within candidate regions and candidate genes, we discuss the results under different classical genetic model assumptions.

**COMT haplotype relative risk for substance dependence in four populations.** *K. Xu<sup>1</sup>, W. Mangal<sup>1</sup>, S. Nagarajan<sup>1</sup>, E. Ferro<sup>1</sup>, X.H. Liu<sup>2</sup>, D. Goldman<sup>1</sup>.* 1) Laboratory of Neurogenetics, National Institute on Alcoholism, Rockville, MD. 20852; 2) Department of Psychiatry, West China University of Medical Science, Sichuan 610041, PR China.

Evidence for a dopaminergic mechanism of reward is found in animal models of substance dependence and in the human [Volkow et al, 1996]. Dopamine also appears critical in prefrontal executive cognition crucial in impulse control. Catechol-O-methyltransferase (COMT) metabolizes released dopamine, as well as other catecholamine neurotransmitters. A common polymorphism, Val158Met, exerts a four-fold effect on enzyme activity and the higher activity Val158 allele which may be associated with lower brain dopamine levels- was associated to poorer prefrontal cognitive executive function in various datasets [Egan et al, 2001, Lipsky et al]. Previously the Val158 allele was also linked to drug abuse [Vandenberg et al, 1997], a result confirmed in a Haplotype Relative Risk study of heroin addicts but not in a subsequent case-control study of such patients. We attempted to replicate the association to substance dependence in large, relatively well-defined Finnish, Southwest Indian, Plain Indian, and Chinese populations, and using genomic control loci to test for stratification, and a multiple locus COMT haplotype consisting of 287G>A, 372C>T, 1243A>G, and 1883G>C, in addition to Val158Met [1947A>G]. 5-exonuclease assays were developed for the five SNPs and 2500 individuals were genotyped. The linkage phenotype was heroin dependence in the Chinese and alcoholism in the other populations. Haplotypes and linkage disequilibrium were compared across populations, revealing population-specific haplotypes and LD relationships. In particular, there was a large reduction of haplotype diversity in the Plains Indians, and multiple haplotype backgrounds were seen for Val158 and Met158 in the other populations. For the Chinese population case/control comparison [N= 450 vs N=304], there was no evidence for association of COMT Val158Met or individual COMT loci to heroin dependence, nor was population stratification detectable using genomic control loci.



**ASSOCIATION OF SPECIFIC IL4 RECEPTOR SNPs WITH TYPE I DIABETES IN FILIPINOS.** *T.L.*

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Over 50% of the genetic predisposition to type 1 diabetes (T1D), a multifactorial autoimmune disorder, has been attributed to the HLA region. Within the HLA region, multiple loci, such as HLA-DRB1, -DQA1, -DQB1, and to a lesser extent, -DPB1 and -A contribute to the genetic risk. Among Filipinos, HLA class II and class I genes also play a critical role in susceptibility to T1D. These patients have been previously characterized for C-peptide levels and for autoantibodies to islet cell autoantigens. We have examined the role of SNPs within a panel of candidate genes, consisting primarily of cytokine and cytokine receptor genes in the Filipino population. Here we report the association of the IL4-R gene with T1D among Filipinos. Eight SNPs (I50V, N142N, E375A, L389L, C406R, S478P, Q551R, and S761P) within the IL4-R locus were examined by comparing the genotype frequencies between patients and controls. In general, homozygotes for the majority allele at these SNPs showed an increased frequency among patients and heterozygotes showed a decreased frequency. For example, the L389L IL4-R SNP (G to T base change)  $p=0.004$ , the frequency of genotype GG is 88% in patients vs. 67% in controls, while the frequencies of genotype GT and TT are 11% vs. 31% and 1% vs. 2%. Moreover, at I50V we found that Ile carriers were 4.16 more likely to be GAD+ than the Valine homozygous ( $p>0.01$ ). The analysis of linkage disequilibrium (LD) patterns revealed that four of these SNPs (E375A, L389L, C406R, S478P) showed very strong LD, revealing two major allelic lineages. An inferred haplotype (complex allele) consisting of primarily of minority SNPs at these sites appeared to confer protection (OR=0.4,  $P=0.01$ ). The minority allele at some IL-4R SNPs have been associated with asthma and atopic allergy, a Th2-mediated disease. We propose that the minority allele at IL4-R may predispose to a Th2-like response and to confer some protection from T1D.

**Application of Haplotype-based Methods to Case-only Studies of Gene-by-Environment Interaction. M.D.**

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Recently, several investigators have proposed using case-only studies to investigate multiplicative gene-by-environment (GxE) interactions using single-locus genotypes. When an assumption of independence between the genetic and environmental factors of interest in the control population is met, this approach can have greater efficiency than a case-control design. We propose a case-only method for assessment of GxE interaction using multi-locus haplotypes. Haplotype-based methods for disease-gene identification have considerable advantage over single-genotype tests due to their ability to identify unique chromosomal segments that harbor disease variants. In light of the high genomic frequency of single nucleotide polymorphisms (SNPs) and the increasing ease of SNP identification and genotyping, we recently developed a SNP-based haplotype frequency testing approach for unrelated case-control data (Fallin et al 2001). We now propose the extension of this method to case-only tests for GxE interaction. Our method estimates haplotype frequencies from multiple SNP genotypes using the E-M algorithm and tests for heterogeneity of haplotype frequencies between cases exposed to the environmental factor and cases not exposed to the environmental factor using a likelihood ratio test. Furthermore, given the likelihood of sparse data when considering observed haplotypes, a random permutation approach to the assessment of empirical p values is employed. The type 1 error as a function of violations from GxE independence among the control population as well as the power of this method under several models of interaction, haplotype or exposure frequencies, and main effect sizes are calculated through Monte Carlo simulations. We show the use of SNP-based estimated haplotypes increases the power of case-only GxE tests in situations where the functional SNP is not typed or where several disease-associated mutations or haplotypes exist.

**Association of TNFA and TNFB polymorphisms with response to oxidative stress in coal miners.** *A.E. Jedlicka<sup>1</sup>, R. Nadif<sup>2</sup>, M. Mintz<sup>1</sup>, J-P. Bertrand<sup>3</sup>, F. Kauffmann<sup>4</sup>, S. Kleeberger<sup>1</sup>.* 1) Dept Environmental Health, Johns Hopkins Univ, Baltimore, MD; 2) INSERM U420, Vandoeuvre, France; 3) Service Medical HBL, Freyming-Merlebach, France; 4) INSERM U472, Villejuif, France.

Genetic factors that modify the response to oxidative stress are largely unknown. The influence of TNFA and TNFB polymorphisms on blood antioxidant enzyme activities was studied in miners exposed to two environmental oxidative stresses: coal mine dusts and smoking. In a longitudinal study, 231 coal miners from the Lorraine Bassin were genotyped for polymorphisms in TNFA (-308 position) and TNFB (first intron). Blood antioxidant enzymes [Cu<sup>++</sup>/Zn<sup>++</sup> SOD, glutathione peroxidase (GSH-Px) and catalase], total antioxidant, and selenium concentrations were measured. Cumulative and current smoking and occupational exposure were recorded. Chest X-rays were scored according to International Labor Office (ILO) classification of pneumoconiosis radiographs. Results indicate GSH-Px activity was decreased in miners heterozygous (1/2) or homozygous (2/2) for the TNFA mutant allele (41.6 v 37.1 U/g hemoglobin (Hb), p=0.03). The greatest difference was in miners with high current exposure (40.6 v 34.0 U/g Hb, p=0.01). In miners with cumulative dust exposure  $\leq 68$  mg/m<sup>3</sup>.y, no difference in GSH-Px activity was found between miners of any TNFA genotype. GSH-Px activity was decreased in miners with genotype 1/2 or 2/2 and cumulative dust exposure >68 mg/m<sup>3</sup>.y [gene-environment (GxE) interaction p=0.05]. No interaction was found with smoking. For TNFB, catalase activity was lower in miners homozygous for the mutant allele (p=0.02). The TNFB polymorphism, smoking and ILO profusion grade were significantly and independently related to catalase activity. GxE interaction (TNFB \* smoking) was observed with the strongest difference in ex-smokers (p=0.01). Interaction with ILO grade was observed; the strongest difference was in miners with ILO 1/1 or more (p=0.07). The conclusion did not change after simultaneously considering smoking and ILO grade. Results show the influence of TNFA and TNFB polymorphisms on the response to biologically measured oxidative stress and interactions with environmental oxidants.

**Comparison of Single-Point and Selected Haplotype-Based Association Statistics for Case-Control Data. S.**

*Lewitzky<sup>1</sup>, R. Martin<sup>1</sup>, K. Rinard<sup>1</sup>, A. Aelony<sup>1</sup>, A. Parker<sup>1</sup>, K. Yu<sup>1</sup>, G. Abecasis<sup>2</sup>, J. Meyer<sup>1</sup>.* 1) Human Genetics, Millennium Pharmaceuticals Inc., Cambridge, MA; 2) University of Oxford, Oxford, United Kingdom.

With the growing availability of information on SNPs throughout the genome, SNPs will undoubtedly play an increasingly important role in case-control association studies. However, many questions remain regarding the optimal choice of analytic methods. Single-point association statistics are relatively easy to compute and interpret, but, unless the functional variant itself is analyzed, they may be less powerful than methods that test for preserved founder haplotypes. However, haplotype-based methods raise numerous questions including how to account for unphaseable and ungenotyped markers, and how to choose appropriate haplotype lengths for analysis.

Using simulated SNP haplotype data for case-control samples, we compare single-point and selected haplotype-based association statistics under a variety of models of disease haplotype preservation and background linkage disequilibrium, assuming patterns of missing genotype data as observed in our actual association studies. We assume no population stratification. For single-point analysis we employ Fisher's Exact Test for a 2x2 contingency table. For haplotype-based analysis, we employ a recently developed EM-based software package (see abstract by Abecasis et al.) to estimate haplotype frequency distributions among cases and among controls. Using these distributions we calculate several haplotype association statistics, and empirically estimate their null distributions by repeatedly sampling case and control haplotypes, with replacement, from a merged haplotype frequency distribution. Some of these statistics are based on differences between case and control haplotype frequency distributions; others are based on within-group haplotypic similarity; still others are based on contrasting within-group similarity to between-group similarity. We compare these statistics for power, false-positive rate, and localizability.

**Association study of the tryptophan hydroxylase gene and bipolar affective disorder in Taiwan.** *H.S. Sun<sup>1</sup>, C.Y. Wu<sup>1</sup>, CathyS.J. Fann<sup>3</sup>, T.M. Chen<sup>1</sup>, T.J. Lai<sup>2</sup>.* 1) Institute of Molecular Medicine, National Cheng Kung University Medical College, Tainan, Taiwan; 2) Department of Psychiatry, Chung Shan Medical and Dental College Hospital, Taichung, Taiwan; 3) Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan.

Bipolar affective disorder (BPD), also known as manic-depressive illness, is a chronic, severe mood disorder characterized by adult onset and episodes of mania and depression. Little is known about the underlying causes of this common and severe illness which has estimated a lifetime prevalence of 0.5% to 1% in various populations. Although the family, twin and adoption studies strongly suggested genetic factors in etiology, searching for predisposing genes by linkage analyses have been fraught with difficulty. Current studies using the candidate gene approach have suggested that serotonergic neurotransmission and metabolism were associated with several psychiatric and behavioral traits including BPD, depression, suicidality, alcoholism, and mental control. Tryptophan hydroxylase (TPH) is the rate-limited enzyme in the biosynthesis of the neurotransmitter serotonin and has been studied extensively for the involvement in the etiology of psychiatric and suicidal behaviors. The objective of this study is to investigate association of the TPH gene and the BPD in Taiwan by candidate gene approach. The allele frequencies of TPH intron 7 polymorphism in 183 unrelated Taiwanese are 0.52 and 0.48 for the "A" and the "C" allele, respectively. A significant association of the TPH polymorphism to Taiwanese bipolar patients was obtained ( $P = 0.03$ ). Furthermore, a marginal association of CC genotype comparing to the AC + AA genotypes with BPD was also obtained ( $P = 0.05$ ). TPH polymorphism was associated with BPD in French. The present study confirms this association in Taiwanese thus suggesting a functional variant(s) in or close to the TPH gene may predispose individuals to bipolar disorder.

**An association of the G Protein Beta 3 Subunit C825T polymorphism and systolic blood pressure in the Old-Order Amish using a regression of offspring on one parent (ROOP) method.** *G.J. Papanicolaou<sup>1</sup>, W. Siffert<sup>2</sup>, P. Platte<sup>3</sup>, A.J. Stunkard<sup>4</sup>, C. Francomano<sup>5</sup>, A.F. Wilson<sup>1</sup>.* 1) Genometrics Section, NIH/NHGRI/IDRB, Baltimore, MD; 2) Dept. Pharmacology, Univ. Hospital Essen, Germany; 3) Cntr. Psychobiol. and Psychosomatic Res., Univ. Trier, Germany; 4) Dept. Psychiatry, Univ. PA, Phil., PA; 5) NIA, NIH, Baltimore, MD.

A number of independent studies have reported an association between the G-protein beta 3 subunit (GNB3) 825T allelic variant and hypertension. As part of an ongoing study of obesity in the Old-Order Amish, the 825T variant was determined in seven families of 157 individuals. Phenotypes measured included height, weight, body mass index (BMI), systolic and diastolic blood pressures, and physical activity. Standard statistical methods were initially used to investigate allelic and genotypic associations between this G-protein variant and quantitative measures of systolic and diastolic blood pressures and BMI. A variation on a new regression-based method (ROMP) of testing for an association between a quantitative trait and a SNP was used to determine if the 825T variant had an effect on obesity-related traits. ROMP is an extension of quantitative genetic theory, and is based on the traditional linear regression of offspring on mid-parent value and on the inclusion of a candidate locus effect as a covariate of the regression. A variation of this method, based on the regression of offspring on one parent (ROOP) was used to estimate the heritability of the traits, test whether the heritability of the trait was greater than zero, test for an association between the SNP and the trait, and estimate the heritability attributable to the SNP. Of the traits considered, the 825T variant had the largest effect on systolic blood pressure. The heritability of systolic blood pressure in this sample was greater than zero ( $p=0.0235$ ) and estimated to be 0.38. The heritability attributed to the 825T variant ( $p=0.053$ ) was estimated to be about 0.04. This suggests that in this sample of the Old-Order Amish the 825T allele was responsible for about 10% of the additive genetic portion of the phenotypic variation in systolic blood pressure.

**A case-control study of selected candidate genes in the Hispanic population of San Luis Valley, Colorado.** *E.J. Parra<sup>1</sup>, C. Bonilla<sup>1</sup>, C.L. Pfaff<sup>1</sup>, S. Dios<sup>1</sup>, A.V. Buchanan<sup>1</sup>, K.M. Weiss<sup>1</sup>, R.E. Ferrell<sup>2</sup>, J.A. Marshall<sup>3</sup>, R.F. Hamman<sup>3</sup>, M.D. Shriver<sup>1</sup>.* 1) Dept Anthropology, Penn State Univ, University Park, PA; 2) Dept Human Genetics, Univ. Pittsburgh, Pittsburgh, PA; 3) Dept of Preventive Medicine and Biometrics, School of Medicine, Denver, CO.

We have carried out a case-control study in a sample of diabetics and controls from the Hispanic population of San Luis Valley, Colorado. We have studied several polymorphisms within five candidate genes previously reported to be associated with type 2 diabetes or related phenotypes in other Hispanic populations (CAPN10, GNB3, PPARG, SUR1 and KCNJ11). Additionally, we have genotyped 14 ancestry informative markers to determine the Native American and European contribution in the sample and to test for the presence of population structure, which could potentially cause false positive results. The Native American genetic contribution in the total sample was estimated to be 37.8%, and the European contribution 62.2%. No significant difference in Native American genetic contribution was observed between the diabetic and control group (38.7% and 36.7%, respectively), and no evidence of the presence of population structure was detected. We have not observed any evidence of association of type 2 diabetes and the CAPN10 gene, which has been recently reported to be the putative diabetes susceptibility gene located in the NIDDM1 region on chromosome 2. No evidence of association was observed for GNB3 and PPARG. Finally, several polymorphisms in the SUR1 and KCNJ11 genes, which code for the two subunits of the beta-cell ATP-sensitive potassium channel and are closely linked on chromosome 11, showed suggestive evidence of association with type-2 diabetes ( $0.01 < p < 0.05$ ). However, further analysis of the data indicates that these polymorphisms are unlikely to have a major effect on the susceptibility to diabetes. This research has been supported in part by grants from NIH/NIDDK (DK53958) and NIH/NHGRI (HG02154) to M.D.S.

**Quantitative Similarity-Based Association Tests Using Population Samples.** *H. Zhao, S. Zhang.* Epidemiology & Public Health, Yale Univ Sch Medicine, New Haven, CT.

Although genetic association studies using unrelated individuals may be subject to bias caused by population stratification, alternative methods that are robust to population stratification such as family-based association designs may be less powerful. Furthermore, it is often more feasible and less expensive to collect unrelated individuals. Recently, several statistical methods have been proposed for case-control association test in a structured population that may be robust to population stratification. In this presentation, we propose a quantitative similarity-based association test (QSAT) to identify association between a candidate marker and a quantitative trait of interest using unrelated individuals. For the QSAT, we first determine whether two individuals are from the same subpopulation or from different subpopulations using genotype data at a set of independent markers. We then perform an association test between the candidate marker and the quantitative trait by incorporating such information. Simulation results based on either coalescent models or empirical population genetics data show that the QSAT has correct type-I error rate in the presence of population stratification, and the power of the QSAT is higher than that using family-based association designs.



Program Nr: 1299 from the 2001 ASHG Annual Meeting

**Correlated Time-dependent Model.** *Q. Yue, G. Bonney.* National Human Genome Center, Howard University, Washington, DC.

It is quite common in genetic analysis to use family based data. The family based data is often correlated by subgroups such as families. Within each subgroup the members may share a common risk probability for some diseases. On the other hand, most diseases are also time dependent. Therefore it is worthwhile to consider a model to deal with correlated and time dependent data in the genetic analysis for family diseases.

In our paper, we try to give and implement such a model to study the genetic basis for type 2 diabetes. The model, which we called Shared Lifetime Risk of failure Model, is based on two distributions: survival distribution and risk distribution. This model is going to be implemented in our software G.E.M.S.

**Development of High-Throughput Genotyping Assays and Their Application to the Study of Tuberculosis**

**Susceptibility.** *A.H.Y. Poon*<sup>1</sup>, *A. Jiménez-Corona*<sup>2</sup>, *M. Palacios-Martínez*<sup>2</sup>, *J. Sifuentes-Osornio*<sup>3</sup>, *A. Ponce-de-León*<sup>3</sup>, *M. Bobadilla*<sup>3</sup>, *M. Kato*<sup>3</sup>, *P.M. Small*<sup>4</sup>, *M.L. García García*<sup>2</sup>, *E. Schurr*<sup>1</sup>. 1) Centre for the Study of Host Resistance, McGill University, Montreal, Quebec, Canada; 2) Instituto Nacional de Salud Pública, Cuernavaca, Mexico; 3) Instituto Nacional de Ciencias Médicas y de Nutrición "Salvador Zubirán", Mexico City, Mexico; 4) Medicine, Stanford University, Stanford, CA, USA.

Tuberculosis, caused by *Mycobacterium tuberculosis*, remains one of the most important infectious diseases worldwide. Tuberculosis has a strong host genetics component and it is widely accepted that tuberculosis susceptibility is a polygenic trait. Hence, we have developed Taqman assays for a number of anti-mycobacterial immune response genes. While Taqman assays are useful for high throughput genotyping of single nucleotide polymorphisms (SNP), the proportion of randomly selected SNPs that can be successfully adapted to Taqman assays is not known. We selected 38 SNPs in 21 immune response genes, encoding cytokines, cytokine receptors and signal transduction molecules. Following a standardized protocol and using a test panel of 80 unrelated individuals Taqman assays were developed, evaluated for their robustness and 25/38 (66%) were shown to produce reliable SNP genotypes. The developed assays are presently being used in a population-based case control study of tuberculosis risk in the Orizaba Health Region located in the state of Vera Cruz in south-eastern Mexico. The incidence of tuberculosis in this area is 42.6/100,000. We have enrolled 216 tuberculosis cases, 212 household controls and 216 neighbourhood controls matched for sex, age and ethnicity. *M. tuberculosis* cultures have been established from all enrolled cases. All tested candidate alleles were found to be in Hardy-Weinberg equilibrium among the three groups. Preliminary tests failed to detect significant associations between the known tuberculosis susceptibility genes NRAMP1, IL12RB1, IL1A and IL1B with the disease phenotype. This finding suggests the presence of population specific susceptibility factors that will be investigated in further detail.

**Involvement of vitamin D receptor and HLA loci in host susceptibility to tuberculosis in the Ache of Paraguay.**

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A number of studies show that host genetics play a significant role in determining susceptibility and resistance to tuberculosis. Research in various areas of the world has shown that multiple loci are usually involved, and that these loci differ by population. Native American populations experience some of the highest rates of tuberculosis in the world, and our work is the first to focus on an indigenous South American group, the Ache of Paraguay. In this project we use molecular biological techniques to examine the potential relationship between tuberculosis susceptibility and the human leukocyte antigen (HLA) and vitamin D receptor (VDR) loci, which have been implicated in tuberculosis susceptibility in other non-Native American populations. Two-hundred eighty-two Ache have thus far been tested for tuberculosis using PCR of IS6110, and of 56 percent of these are positive. These preliminary results correlate highly with PPD skin tests and indicate that Ache suffer high prevalence of tuberculosis. Preliminary HLA tests at the DQB1 locus on 14 individuals show that the most prevalent allele is 0302,0307, with 6 of those tested carrying least one copy of the allele. One individual also carries allele 0615, and two others have 0402. Six remaining individuals appear to have new alleles not detected by the kits we are currently using. These early data show that Ache are a highly endogamous population, and as such, provide a unique opportunity to examine the effects of genetic differences among related individuals on susceptibility/resistance to tuberculosis.

**Genetic transmission of tuberculosis-related traits.** *C.M. Stein<sup>1</sup>, R. Mugerwa<sup>3</sup>, P. Peters<sup>2,3</sup>, J. Elner<sup>2</sup>, R.C. Elston<sup>1</sup>, H.K. Tiwari<sup>1</sup>, C.C. Whalen<sup>1,2</sup>.* 1) Epidemiology & Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) Tuberculosis Research Unit, Case Western Reserve University, Cleveland, OH; 3) Makerere University School of Medicine, Kampala, Uganda.

Though the underlying cause of tuberculosis (TB) is bacterial, susceptibility to TB may be influenced by genetic factors. To investigate possible intermediate phenotypes for TB, we analyzed a set of cytokine profiles, which were chosen based on the current biologic model for TB. These traits included antigen-stimulated whole blood assays for interferon-g (IFN), tumor necrosis factor-a (TNF), transforming growth factor-b (TGF), and the ratio of IFN to TNF. We analyzed these data in 285 pedigrees from a community in Uganda with a high prevalence of TB. These intermediate phenotypes were adjusted for previous BCG vaccination, tuberculin skin test reactivity, sex, HIV status, body mass index, and age, to reduce confounding from environmental factors. Estimates for heritability of these traits were greater than 14%, and TNF in particular had an estimated heritability of 68%. Analyses adjusting for tuberculin skin test reactivity and disease status yielded similar results. Because of the high heritability of TNF, we conducted a segregation analysis of this trait, which suggested a major gene effect on TB. A principal components analysis of IFN, TNF, and TGF reflected the immunologic model of TB. In this analysis, the first component explained more than 38% of the variation in the data. Our analysis illustrates the value of such intermediate phenotypes in mapping susceptibility loci for TB.

**Epigenetic heterogeneity at imprinted loci in normal populations.** *T. Sakatani*<sup>1,2</sup>, *M. Wei*<sup>3</sup>, *M. Katoh*<sup>1</sup>, *C. Okita*<sup>1</sup>, *D. Wada*<sup>1</sup>, *K. Mitsuya*<sup>1</sup>, *M. Meguro*<sup>1</sup>, *M. Ikeguchi*<sup>4</sup>, *H. Ito*<sup>2</sup>, *B. Tycko*<sup>3</sup>, *M. Oshimura*<sup>1</sup>. 1) Department of Molecular Cell & Genetics, School of Life Science, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan; 2) First Department of Pathology, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan; 3) Department of Pathology and Institute of Cancer Genetics, Columbia University College of Physicians and Surgeons, New York, USA; 4) Department of Surgery I, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan.

Genomic imprinting is the phenomenon by which the two alleles of certain genes are differentially expressed according to their parental origin. Since relaxation or loss of monoallelic expression of these genes is found in many human hereditary disorders and in tumors, imprinted genes are thought to influence normal embryonic growth, development and behavior. However, despite suggestive evidence, it has not been clarified if the allelic expression patterns of imprinted genes varies within the normal population. In the present study, to establish a standard for allelic expression status of imprinted genes in a general population, we collected peripheral blood leukocyte samples from 262 Japanese normal individuals in 68 families and analyzed for the expression status of *IGF2*, *SNRPN* and *IMPT1* (*TSSC5/ORCTL2/BWR1A/ITM*) genes. By RFLP analysis, the parental origin of alleles was determined for 38 cases for *IGF2*, 36 cases for *SNRPN* and 34 cases for *IMPT1*. In all cases, *SNRPN* was expressed exclusively from the paternal allele, but paternal allele-specific expression of *IGF2* was relaxed in some cases. In all informative cases, maternal allele-specific expression of *IMPT1* was relaxed, and each individual exhibited a distinct quantitative allelic bias. In additional nine cases, the allelic expression bias of *IMPT1* remained constant when re-examined after six months. Our findings add to the accumulating evidence for variable allelic expression at multiple loci in a normal human population. This epigenetic heterogeneity can be a stable trait, and it is possible that it will influence individual phenotypes. This work was supported by CREST of Japan Science and Technology Corporation (JST).

**Complex segregation analysis of ventilatory threshold measured before and after a 20-week endurance exercise training program: the HERITAGE Family Study.** *M.F. Feitosa<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. Bouchard<sup>2</sup>, D.C. Rao<sup>1</sup>, S.E. Gaskill<sup>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) School of Kinesiology and Leisure Studies, 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) Department of Health and Human Performance, Human Performance Laboratory, University of Montana, MT.

Familial aggregation has been demonstrated for maximal oxygen uptake ( $VO_{2max}$ ), both in the sedentary state and in response to exercise training. This study investigates the presence of a major gene for  $VO_2$  at the ventilatory threshold ( $VO_{2vt}$ ) and the proportion of  $VO_{2vt}$  to  $VO_{2max}$  ( $VT\%VO_{2max}$ ), at baseline and in response to 20-weeks of exercise training using data from 336 White subjects (100 pedigrees) and 169 Black subjects (111 pedigrees), from the HERITAGE Family Study. Effects of age, weight, fat mass and fat free mass have been removed separately in each of eight sex by generation by race groups, using stepwise regression procedures. Segregation analysis was performed using PAP, including investigations of genotype-specific effects of covariates. There is strong evidence of a major gene, with frequencies of 0.175 and 0.141 in the upper distribution that account for 52% and 43% of the variance in baseline  $VO_{2vt}$  and  $VT\%VO_{2max}$ , respectively, in White families. Genotype-specific-covariate effects were not significant. However, the segregation results were inconclusive for the responses to training in White subjects, as well as for baseline and training response in Black subjects, probably due to insufficient power because of reduced sample sizes. The consistent genetic signals for  $VO_{2vt}$  and  $VT\%VO_{2max}$  suggest that these should be investigated in further genetic analysis using linkage and association studies.

**Evidence for inherited susceptibility to common colorectal cancer.** *G.P. Crockford<sup>1</sup>, J.H. Barrett<sup>1</sup>, F.T. McDermott<sup>2</sup>, E.A. Debney<sup>2</sup>, E.S. Hughes<sup>2</sup>, D.J.S. St John<sup>2</sup>, D.T. Bishop<sup>2</sup>.* 1) Genetic Epidemiology Division, Imperial Cancer Research Fund, Leeds, W. Yorks, England; 2) Dept of Gastroenterology, The Royal Melbourne Hospital, Melbourne, Australia.

We investigate the evidence for an inherited susceptibility to colorectal cancer (CRC) taking account of families with Mismatch Repair (MMR) mutations in a series of 525 probands and their first and second degree relatives. Four families were known to have MMR germline mutations, 21 had an extended family history of CRC, suggestive of Hereditary Non-Polyposis Colorectal Cancer (HNPCC); 5 fulfilled the Amsterdam II criteria for HNPCC. Segregation analysis under a liability model was performed using PAP (Hasstedt 1994) to fit single gene, polygene and mixed models. Analyses were performed on (i) all 525 pedigrees, (ii) the 521 without known MMR mutations and (iii) data sets with random removal of families based on their probability of having MMR. Results on the 521 families are broadly representative of all analyses. Single major gene models found evidence for a rare, dominantly inherited predisposition to CRC, allele frequency of 0.023 and lifetime risk of 19% (to age 80 years) in gene carriers and 2% to non-carriers. Under the mixed model a common dominant gene, allele frequency 0.33, 33% heritability and lifetime risk of 5% to gene carriers and zero to non-carriers, was the preferred model. The rare dominant model was rejected ( $p < 0.001$ ). Goodness of fit tests obtained by simulation found that the mixed model best approximates the total number of affected relatives while the rare dominant model better predicts the number of families with more than 2 affected relatives. The mixed dominant and the rare dominant models provide good estimates of risk of disease to relatives, with the rare dominant model best able to distinguish differences in risk by age of proband. We conclude that having taken account of MMR genes in this data set there is still evidence for an inherited susceptibility to CRC which is best modelled by a common dominantly inherited gene and polygenic variance. References Hasstedt S J, PAP Manual revision 4 1994, Dept. Human Genetics, U of Utah.

**A putative codominant gene and smoking account for cancer susceptibility in families of pancreatic cancer**

**patients.** *J.F. Korczak<sup>1</sup>, J.S. Barnholtz-Sloan<sup>1</sup>, M. Schenk<sup>1</sup>, S.H. Bunner<sup>1</sup>, M. Kinnard<sup>2</sup>, A.G. Schwartz<sup>1</sup>, D.H. Garabrant<sup>3</sup>.* 1) Karmanos Cancer Inst and Wayne State Univ, Detroit, MI; 2) Case Western Reserve Univ, Cleveland, OH; 3) Univ of Michigan, Ann Arbor, MI.

Pancreatic cancer (PC) is the fourth leading cause of cancer-related mortality in the USA (ACS, 2001). We recently reported familial aggregation of PC in first-degree relatives of 247 newly diagnosed PC patients ascertained through hospitals, physicians, and tumor registries in southeastern Michigan. The relatives' smoking history was also found to be a significant risk factor (Schenk et al., 2001). Additional preliminary analysis suggested an excess of lymphoma (L) and ovarian cancer (OC) in the relatives.

To determine whether transmission of a putative disease gene could account for the observed familial aggregation, we considered anyone with PC, L, or OC as affected and performed segregation analysis using Class A logistic regressive Models 1 (variable age of onset) and 2 (variable susceptibility), implemented in the computer program S.A.G.E. REGTL. For each model, hypotheses of genetic, environmental, or no transmission were tested against a general model with arbitrary transmission probabilities. Analyses were performed either with or without smoking (ever/never) as a covariate, assuming Hardy-Weinberg proportions.

According to the Akaike Information Criterion (AIC), Model 1 fit the data as well as or better than Model 2 for all transmission hypotheses, regardless of whether smoking was included. However, without smoking, all Model 1 transmission hypotheses were rejected, compared to the general model. Inclusion of smoking in Model 1 led to the rejection of all hypotheses except that of codominant inheritance, which provided the most parsimonious fit to the data according to AIC. For this model, the frequency of the disease allele, A, was 0.136, the mean ages of onset were 27.8, 66.1, and 85.2 years for genotypes AA, AB, and BB, respectively, and the cumulative incidence of PC, L, or OC to 80 years of age was 3.6%

These findings may provide a foundation for gene mapping studies in familial pancreatic cancer.



**B-CLL: Familial aggregation and anticipation.** *S.S. Strom, Y. Gu, M. Selvan, F. Chan, M.J. Keating.* The University of Texas M.D. Anderson Cancer Center, Houston, TX.

The etiology of CLL is mainly unknown although studies suggest that both environmental and genetic factors exist. To analyze familial aggregation we conducted a family study of 577 consecutive B-CLL patients registered at M. D. Anderson between 1990 and 1999. Detailed history of all cancers was obtained for 3800 first degree relatives (FDR). A random sample of 25% hematological cancers was verified by medical records. The observed number of cancers in the FDR was compared to the expected number in the general population using Connecticut Cancer Registry age-, sex- and calendar years-specific incidence rates. A small increase in overall cancer was found. The standardized incidence ratio for all invasive cancers among FDR was 1.1 [observed (O)/expected (E)=538/487, 95% confidence interval (CI)= 1.10-1.20]. There were 102 hematological cancers reported: 39 CLL, 11 HD, 23 NHL, 7 multiple myeloma and 22 leukemias. A significant excess was found for CLL among 1380 siblings (O/E= 12/0.98=12.28, CI=6.34-21.46), 572 fathers (O/E=17/1.6=10.59, CI=6.16-16.95) and 573 mothers (O/E= 10/1.18=8.44, CI=4.04-15.53). We identified 21 sib-sib pairs. The risk of developing CLL in FDR was 2.4 times higher among relatives of patients diagnosed before age 55. We analyzed anticipation (decreased age of onset from one generation to the next) in the 26 pedigrees with cases of CLL in two generations. The mean age of diagnosis in the older generation was 64 (SD=12.8 ) years and 50 (SD=6.4) in the younger one. Using the Kaplan-Meier method, the age difference between the survivor functions was highly significant (log-rank test: p, 0.0001).

In summary, familial aggregation, young age of onset, and anticipation in parent-offspring pairs might contribute further insight into the role that genetics plays in the etiology of CLL. Molecular characterization of predisposing genes will be necessary to understand the role of anticipation in CLL. Supported by the Wolf Creek program.

**b-cell dysfunction is heritable in Latino families of probands with gestational diabetes.** *R.M. Watanabe<sup>1</sup>, C.D. Langefeld<sup>2</sup>, A.H. Xiang<sup>1</sup>, E. Trigo<sup>1</sup>, S. Hernandez<sup>1</sup>, F. Berrios<sup>1</sup>, T.A. Buchanan<sup>1</sup>.* 1) USC Keck School of Medicine, Los Angeles, CA; 2) Wake Forest University School of Medicine, Winston-Salem, NC.

We have shown that Hispanic women who develop gestational diabetes (GDM) during pregnancy have a specific b-cell phenotype characterized by a propensity to fail in the presence of chronic insulin resistance and is predictive of type 2 diabetes. We are recruiting and phenotyping Latino families consisting of a GDM proband and her non-diabetic siblings. Phenotyping includes oral (OGTT) and tolbutamide-modified intravenous (IVGTT) glucose tolerance tests, and body composition by DEXA. IVGTT data are analyzed using Bergman's Minimal Model to derive insulin sensitivity ( $S_I$ ). b-cell function is assessed as incremental 30-minute insulin during the OGTT (30'dINS) and incremental area under the insulin curve during the first 10 minutes of the IVGTT (acute insulin response, AIR). We also compute the disposition index (DI), a measure of the ability of the b-cell to compensate for insulin resistance, as the product of AIR and  $S_I$ . Heritability ( $h^2$ ) was estimated using the variance components approach adjusting for age, gender, and BMI. To date, our sample consists of 40 males and 91 females in 31 nuclear families with an average sibship size of 4.2 (range 2-10). All individuals were non-pregnant and had fasting plasma glucose <126 mg/dl at time of testing. Mean ( $\pm$ SD) age was  $35\pm 9$  yrs and BMI was  $29\pm 6$  kg/m<sup>2</sup>. We observed strong  $h^2$  for AIR ( $72\%\pm 23$ ;  $p<0.0001$ ) and DI ( $56\%\pm 25$ ;  $p=0.005$ ) and significant  $h^2$  for 30'dINS ( $40\%\pm 20$ ;  $p=0.021$ ).  $h^2$  for insulin resistance as measured by  $S_I$  ( $33\%\pm 24$ ;  $p=0.09$ ) or homeostasis modeling (HOMA;  $8.7\%\pm 15$ ;  $p=0.52$ ) was not significant. Both BMI ( $78\%\pm 24$ ;  $p<0.00001$ ) and total body fat ( $48\%\pm 16$ ;  $p=0.0002$ ) exhibited strong heritability. Removing BMI as a covariate in the analyses of insulin sensitivity and secretion did not alter the results. The significant  $h^2$  for two independent measures of insulin secretion and a measure of b-cell compensation for insulin resistance suggest there are genetic determinants of the b-cell defect that predicts diabetes in our patients. Our findings support a genetic approach to identification of the fundamental cause(s) of this defect.

**Complex segregation analysis of obsessive-compulsive disorder in Mexican pedigrees.** *K.A. Weissbecker<sup>1</sup>, M.I. Green-Leibovitz<sup>1</sup>, B. Camarena<sup>2</sup>, G. Rinetti<sup>2</sup>, J. Ezzell<sup>1</sup>, D.K. Winstead<sup>1</sup>, H. Nicolini<sup>2</sup>.* 1) Dept. of Psychiatry and Neurology and Hayward Genetics Program, Tulane Med Ctr, New Orleans, LA; 2) Instituto Mexicano de Psiquiatría, Mexico City, Mexico.

Obsessive-Compulsive Disorder (OCD) is characterized by obsessions (intrusive and persistent ideas or thoughts) and compulsions (senseless, repetitive purposeful behaviors) which interfere with normal functioning. We explored the mode of inheritance of OCD and the effect different definitions of the affected phenotype had on the outcome of the analyses. Seventy-seven OCD patients were ascertained at the Instituto Mexicano de Psiquiatría, in Mexico. Psychiatric diagnoses were obtained from 523 relatives either by direct interview or the family history method. We performed a segregation analyses under class A logistic regressive models using the REGTL computer program (S.A.G.E.). Model 1 of REGTL, which presumes genotype (or "type") influences the age of onset of the disease state was implemented. Relatives of the probands were classified as affected by two phenotypic definitions and separate analyses were run. Under the narrow definition, only relatives with definite OCD were classified as affected (N= 34). The broader definition also classified relatives with "sub-clinical" OCD as affected (N=59). Individuals with sub-clinical OCD have excessive obsessions and/or compulsions, but there is little interference with normal functioning. Using the narrowly defined affected phenotype, the sporadic, all environmental and all Mendelian models were rejected when compared the unrestricted models which estimated transmission probabilities. Under the broad phenotype, the sporadic and environmental models were rejected, but not the Mendelian models. The codominant or arbitrary models, which allow for three age of onset distributions, did not significantly improve the likelihood of the data. The most parsimonious model was autosomal dominant with a very low disease allele frequency and a mean age of onset of 15.42 for the AA and AB genotypes with 57% penetrance. These analyses confirm the presence of a major gene in the etiology of OCD when sub-clinical OCD is included in the disease phenotype.

**Familial clustering of nephropathy in hereditary amyloidosis TTR Val30Met.** *J. Sequeiros<sup>1</sup>, L. Lobato<sup>1, 2, 3</sup>, I. Beirao<sup>2, 3</sup>, M. Silva<sup>2</sup>, P. Costa<sup>3</sup>, S. Guimaraes<sup>2</sup>, A. Sousa<sup>4</sup>.* 1) UnIGENe - IBMC, Univ Porto, Porto, Portugal; 2) Dept Nephrology, Hosp Santo Antonio, Porto, Portugal; 3) CE Paramiloidose, INSA; 4) Dept Pop Studies, ICBAS, UP.

Familial amyloid polyneuropathy type I, FAP, is due to a point mutation in the transthyretin gene (TTR Val30Met) and is inherited as an autosomal dominant. It manifests mainly as a peripheral neuropathy; renal disease can be present. We now studied the frequency and familial predisposition to kidney disease in FAP. A prospective survey in the 3 main regions of FAP in Portugal was conducted in 1990-2000. In the same period, FAP patients from other areas who developed end-stage renal disease (ESRD) and their affected relatives, were also followed. Proteinuria  $>0.3\text{g/l}$  or renal insufficiency (creatinine  $>1.2\text{mg/dl}$  or serum urea  $>50\text{mg/dl}$ ) were criteria for nephropathy, and proband the first ESRD patient in the family; 496 patients (247M/249F), 165 families, were evaluated. Nephropathy was present in 194 patients (87M, 107F), 39% FAP subjects studied, involving 86 kindreds. ESRD was found in 33 families, 47 (15M, 32F), 9.4% of all FAP patients and 24.2% of nephropatic. Outside the main areas we registered 27 kindreds with 34 ESRD patients. We ascertained 60 probands, and 276 patients in their families; 81 of the 158 patients with overt nephropathy had ESRD. In any area, there was a higher frequency of clinical nephropathy in families of probands, than in those without patients progressing to ESRD (46% vs 28%,  $p<0.01$ ); 32 probands had sibs on the study. In sibships of probands, 78% had renal features, half with ESRD. Among first-degree relatives, 56.4% presented renal features. Sibs had a higher proportion of ESRD ( $p<0.002$ ) compared to other 1st-degree relatives, a OR 5.3 (CI 95%: 1.716). In conclusion, (1) nephropathy was present in 1/3 patients from classical areas of FAP; (2) affected relatives, and particularly sibs, of ESRD patients were more prone to develop nephropathy. This familial aggregation of renal disease in FAP suggests the influence of additional genetic factors.

**Linkage disequilibrium maps of human chromosomes.** *A. Collins, N. Maniatis.* Human Genetics, Univ Southampton, Southampton, England.

Positional cloning of disease genes depends on linkage and allelic association (also called linkage disequilibrium or LD). Neither the linkage nor LD map is proportional to the sequence-based map. To the degree that LD reflects recombination it can extend the low resolution of the linkage map (a region with extensive LD might coincide with a recombination cold spot). We need an LD map to facilitate positional cloning, extend the resolution of the linkage map, compare populations and detect selective sweeps and other events of evolutionary interest. A key property of a chromosome map, whether physical or genetic, is that its distances are additive and we require a standard LD map to which population specific maps are roughly proportional with deviations that mirror the effects of gene frequency, selection, mutation, time and drift. The Malecot prediction of association ( $r$ ) is  $(1-L)Me^{-ed}+L$ , where  $L$  is the bias at large distance,  $M$  reflects mono or polyphyletic origin and  $e$  is the exponential decline of  $r$  with distance  $d$ . A natural measure for the length of the  $i^{\text{th}}$  map interval is  $e_i d_i$  where  $e_i$  estimates the Malecot parameter when the two markers that flank the interval are paired with all other markers and  $d_i$  is the length of the interval on the physical map in Kb. The minimal error variance from the regression of  $e_i d_i$  on the sex-averaged linkage map determines the optimal estimator of  $e_i$ . We consider here a number of ways to establish additivity of LD distances. Given an optimal LD map at higher resolution than the linkage map the LD information may be integrated within adjacent reference markers. Alternative approaches to establishing an LD map can be evaluated by comparing fit to the Malecot model. The optimal LD map, however obtained, should fit better than either the linkage map and, particularly, the physical map. Sequence-based maps that integrate sex-specific linkage maps have been created for human chromosomes 21 and 22. For LD mapping we used version 9 of the CEPH database. The marker spacing is near the limit for LD mapping but, for certain chromosome regions, is very useful for evaluation of alternative approaches. We present here our findings for these chromosomes.

**A simple genotype-quantitative phenotype relationship in humans: a case study of T cell receptor Vbeta6.7.** *K.R. Ahmadi<sup>1,2</sup>, M. Hall<sup>2</sup>, P.J Norman<sup>3</sup>, R. Vaughan<sup>3</sup>, T.D Spector<sup>1</sup>, J.S Lanchbury<sup>2</sup>*. 1) Twin Research & Genetic Epidemiology Unit, St.Thomas' Hospital, UK; 2) Molecular Immunogenetics Unit, Department of Rheumatology, GKT School of Medicine, King's College London; 3) South Thames Tissue Typing, Guy's Hospital, London, UK.

The success achieved in mapping monogenic disease genes has failed to carry over to diseases with complex etiology. Although sparse data exist, most of our knowledge concerning the relationship between an underlying genotype and its dependent quantitative phenotype has either come from model animal systems (bristle counts in *Drosophila*) or over-simplified population simulation studies.

The monoclonal antibody OT145, recognises an allotypic variant of the T cell antigen receptor beta chain variable region called VB6.7. Discounting factors such as experimental error and random noise, quantitative variation in the OT145-recognised epitope should be largely genetically determined. A 15-allele short tandem repeat (STR) marker is located in the first intron of the TCRVB6.7 gene. We measured VB6.7 as a quantitative trait and typed the VB6.7-associated STR in a sample of 45 identical and 73 non-identical female Caucasoid twin pairs.

The heritability of VB6.7 level was 86%. Linkage of the STR to VB6.7 wasn't detected, but the omnibus test of association was highly significant ( $p=1 \times 10^{-9}$ ). However, the association parameter only accounted for 24% of the total variance in VB6.7, leaving 62% of the variance unaccounted for. Through simulations, we show that the QTL heritability can only be fully represented by a marker that is in perfect linkage disequilibrium (LD) with the QTL, a measure which is independent of sample size. These results show that even for homogenous traits with high heritability and theoretically perfect genotype-phenotype correlation, association mapping of quantitative traits can be problematic. For the attributable effect to be quantified accurately with small sample sizes, sufficient LD must exist between marker and QTL alleles which must be in phase and comparable in frequency.

**Molecular analysis of haplotype diversity associated with haemoglobin E in Vietnam.** *R.K Basran, J.B Clegg, A.J Boyce, R.M Harding.* Institute of Molecular Medicine, University of Oxford, Oxford, England.

Haemoglobin E (b26 glu—>lys) is one of the most common haemoglobin variants in Southeast Asia. Its high frequency is probably due to the protection it confers against malaria. The frequency of Hb E in the S'Tieng, an ethnic minority group located in southern Vietnam with origins in Thailand, is 45%. The main aim of this study is to investigate the role of recombination and gene conversion in generating haplotype diversity associated with Hb E. The absence of Hb E in Africa and the Mediterranean where malaria is endemic suggests that the contribution to haplotype diversity of independent recurrent Hb E mutations is minimal.

Seven RFLPs distributed within 63 Kb of the b-globin gene cluster on chromosome 11 have been typed to determine the haplotypes of 210 Hb E chromosomes, all from individuals homozygous for Hb E. We have also inferred 62 haplotypes from normal chromosomes in individuals heterozygous for Hb E. Between five RFLPs (the 5' sub-haplotype) and two RFLPs flanking the b-globin gene (the 3' sub-haplotype) is a well characterized hotspot for recombination. We see different distributions of haplotypes associated with the normal and Hb E chromosomes. 67 % of the Hb E chromosomes are associated with the +/- 3' sub-haplotype, which only comprise 10% of the normal chromosomes. Hb E on the +/- 3' sub-haplotype associates with five different 5' sub-haplotypes, reflecting recombination at the hotspot. Of these, three haplotypes are common among Hb E chromosomes, but only one is common among normal chromosomes. Hb E is also found in association with the other three possible 3' sub-haplotypes (-/+, -/-, +/+) probably through gene conversion. We are also investigating the extent to which linkage extends from the hotspot (both 5' and 3') by typing SNPs up to 5 Mb away in individuals who have homozygous Hb E haplotypes. A panel of SNPs from the SNP Consortium database have been tested in the S'Tieng and only 37% of the characterized SNPs were found to be polymorphic in this population. Preliminary results indicate that linkage extends up to 1 Mb away from the mutation.

**Homozygosity of the prion Met129Val polymorphism increases risk of developing clinical early-onset but not late-onset Alzheimer's disease.** *B. Dermaut<sup>1</sup>, E. Croes<sup>2</sup>, M. Cruts<sup>1</sup>, M. Van den Broeck<sup>1</sup>, C.M. van Duijn<sup>1,2</sup>, C. Van Broeckhoven<sup>1</sup>.* 1) Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), University of Antwerp, Antwerpen, Belgium; 2) Department of Epidemiology and Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands.

The clinical distinction between familial early onset Alzheimer's disease (AD) and familial Creutzfeldt-Jakob disease (CJD) is sometimes difficult. In some cases, histopathological hallmarks of both disorders are observed simultaneously. Moreover, familial aggregation of CJD with dementia due to other causes than CJD has been reported repeatedly. These observations suggest that common pathogenic factors may underly both CJD and other types of dementia, including AD. As homozygosity of the Met129Val polymorphism in the prion protein gene (PRNP) is a well established genetic risk modulator of all types of human prion diseases, we investigated the role of this locus on the risk for developing AD. In two Dutch population based series of early (n=122) and late-onset AD (n=245), genotype frequencies of the PRNP Met129Val polymorphism were compared to an age matched control group (n=287 and n=244 respectively). Results were corrected for age and gender using logistic regression. Genotype frequencies were in Hardy-Weinberg equilibrium in both control groups ( $p > 0.2$ ). While we found no effect of the Met129Val polymorphism on the risk of late-onset AD, we observed a statistical significant influence of Met129Val in the group with early-onset AD. Using the Met/Val genotype as a reference, homozygosity resulted in a 1.81 times increased risk (95% CI 1.10-2.98;  $p = 0.02$ ). In Val homozygotes the risk of developing early-onset AD was 2.43 times increased (95% CI 1.15-5.11;  $p = 0.02$ ). These results suggest that PRNP Met129Val is genetically associated with clinically diagnosed early but not late-onset AD.



**Gene-environment interactions and linkage disequilibrium in familial analysis of complex diseases.** *V. Chaudru, M. Rosenberg, F. Demenais.* INSERM EMI 00-06, Evry, France.

Investigating gene-environment (GxE) interactions in chronic diseases is of interest once a disease gene has been mapped to a region where there is linkage disequilibrium (LD). Our goal was to assess by simulations : 1) how LD can influence the power to detect GxE, 2) how ignoring GxE can affect detection of LD.

Affection status, environmental factor and marker data were simulated in 165 nuclear families of varying size. The liability to the disease was generated under a model including a disease gene interacting with an environmental factor, a polygenic component and random environment. The proportion of total variance due to each component was : 15% for the gene, 15% or 25% for GxE component, 30% or 35% for the polygenic component, the remaining being due to random environment. The disease prevalence was 10%. Four models were considered for the disease gene : 2 dominant models with allele frequency ( $q$ ) equal to 0.1 or 0.3 and displacement between homozygous means,  $t$ , of 1 or 0.78 and 2 recessive models with  $q$  being 0.3 or 0.5 and  $t$  equal to 1.35 or 0.99. We generated a tightly linked SNP with allele frequencies equal to the disease gene frequencies and varying LD with that gene : no LD ( $D/D_{max}=0$ , where  $D$  is the measure of LD), CLD ( $D/D_{max}=1$ ), ILD ( $D/D_{max}=0.5$ ). One hundred replicates of family samples were generated for each set of parameters. Segregation-linkage analyses of the simulated data were conducted using the regressive threshold model.

When there is CLD, power to detect GxE is greater than 90%. When there is ILD, the power to detect GxE is only reduced to 90% when  $q < 0.3$  and  $t > 1$ . However, this power is decreased to 60% when  $q = 0.5$  with weak GxE and drops to 40-50% when  $t < 1$ . When there is no LD, the power decreases to 50%-75% for  $q < 0.3$  and  $t > 1$  and to 20-35% when  $q = 0.5$  or  $t < 1$ . Alternatively, ignoring an existing GxE in the analysis may affect the detection of the true LD model : CLD is correctly detected in  $> 70\%$  of replicates while ILD is supported in 40-60% of cases. Taking into account both GxE and LD appears of importance in analysis of complex diseases.

**The 4002AtoG polymorphism of the CFTR gene can interfere with the detection of the common 1282X and 3905insT mutations that occur within exon 20: Implications for carrier screening.** *Y.K. Goldberg<sup>1</sup>, S. Brown<sup>2</sup>, S. Nakagawa<sup>1</sup>, L.H. Kellner<sup>1</sup>, S.J. Gross<sup>1</sup>, H.M. Nitowsky<sup>1</sup>.* 1) Dept Ob-Gyn; Montefiore Medical Ctr, Bx, NY; 2) Dept Ob-Gyn; Columbia University NYC, NY.

Population screening for carriers of cystic fibrosis mutations (CFM) is becoming increasingly common, and a variety of molecular strategies for the detection of CFM in the CFTR gene have been developed. A multiplex PCR/oligonucleotide ligation assay (Applied Biosystems(ABI)), allows for the simultaneous detection of 31 common CFM. In the course of screening approx. 3600 persons, we have noted that in 0.3% the amplicon containing exon 20 fails to amplify. Individuals in whom exon 20 failed to amplify were shown to be homozygous for the previously described 4002AtoG polymorphism that occurs within exon 20. Although the polymorphism is silent and presumably neutral, its presence evidently interferes with PCR amplification within the context of the ABI CFM assay. Failure to amplify is easily detected in homozygous (PCR products are absent) but presumably remains undetected in heterozygotes. Therefore, CFM occurring in cis with the polymorphism will not be detected using this assay. Hardy-Weinberg equilibrium predicts that about 10% of all persons in our population are carriers of the 4002AtoG polymorphism. This is significant since the 1282X CFM, which is present in about 1/40 (2.5%) of Ashkenazi Jewish (A.J.) individuals, occurs within exon 20. The extent to which carriers of the 1282X CFM are misdiagnosed as normal with the ABI assay depends on the degree to which there is linkage-disequilibrium (LD) between 1282X and 4002AtoG. We studied the frequency of the 4002AtoG polymorphism in our A.J. population. In a series of 137 persons, we found that 10 are heterozygous for the 4002AtoG polymorphism as determined by PCR amplification followed by restriction digestion. This indicates that our prediction of heterozygote freq. for the 4002AtoG polymorphism (based on the number of persons failing to amplify exon 20 with the ABI assay) appears to be correct and needs further investigation. We are now determining the degree to which there is LD between 1282X and 4002AtoG.

Program Nr: 1317 from the 2001 ASHG Annual Meeting

**Linkage disequilibrium in human populations and the design and interpretation of association studies.** *D.B. Goldstein, M. Weale, C. Capelli, J. Wilson.* Biol/Galton Lab/Wolfson House, Univ Col London, London, England.

The appropriate design and interpretation of association studies requires detailed information about the patterns of linkage disequilibrium (LD) in human populations. Here we use published and unpublished data to assess the distribution of LD as a function of genetic distance between sites in multiple genomic regions in multiple Eurasian populations, focusing attention on the implications for study design and interpretation. We also describe a quantitative framework for assessing the expected power of association studies based on the observed distributions of LD.

**Fine-mapping strategies using sequential optimal parameter selection.** *R.B. Martin, K. Yu, S. Lewitzky, A. Parker, J. Meyer.* Human Genetics, Millennium Pharmaceuticals Inc, Cambridge, MA.

For complex disorders, linkage genome scans typically are able to localize a disease susceptibility locus to within a region of around 20-30cM. Once such a region has been identified, fine-mapping often proceeds using linkage disequilibrium (LD) analysis applied on a series of increasingly dense marker maps. However, it remains unclear for general situations how marker location, allele frequency, and number of markers can be chosen to optimize this fine-mapping process.

We propose a fine-mapping strategy that can be used in conjunction with single-point LD methods by describing marker choice as a sequential optimal parameter selection problem. Using linkage information as a prior to guide marker location, an initial set of markers is selected and analyzed, and significance levels are corrected for multiple testing taking into account observed gametic disequilibrium. The algorithm terminates if either a stopping criterion involving significance is met or if the number of iterations becomes too large, else it repeats with an expanded set of markers. Marker selection may be controlled both by linkage disequilibrium estimates calculated during the process and by initial linkage data.

The aim is to maximize the probability of stopping within a small region surrounding the disease susceptibility locus, in which maximal LD exists due to the presence of untrimmed ancestral haplotypes descended from a common founder. By optimizing marker selection and employing a sequential testing strategy, we hope to minimize the number of markers used and thus avoid multiple testing problems that could make it difficult or impossible to detect the disease susceptibility locus. The method is benchmarked using simulated data based upon a study of schizophrenia in a Finnish population.

**Association analysis: within-sibship sampling variation and solutions.** *C. Li, M. Boehnke.* Dept Biostatistics, Univ Michigan, Ann Arbor, MI.

In classical case-control studies, we sample independent affected and unaffected individuals and tally their alleles (or haplotypes) into a  $2 \times k$  table, where  $k$  is the number of alleles. We then test for independence between the alleles and affection status, or equivalently, for equal distribution of alleles in the affected and unaffected groups.

Often we sample affected siblings and other relatives of the affected individuals to do linkage analysis for the disease of interest. The affected relatives' information is not used in case-control studies, resulting in inefficient use of data.

We propose two methods to use efficiently genotype data of affected sibships. One is to count all alleles of an affected sibship, but down-weight the sibship so that its total allele contribution is 2. The relative efficiency of this method vs. the standard one is 1.3 for sib pairs and 1.5 for sib trios. The Pearson's chi-squared test can be performed as usual. We also introduce a likelihood ratio statistic and a permutation test.

Another method is to down-weight an affected sibship of size  $k$  so that its total contribution is  $4k/(k+1)$  (Broman 2001 *Genet Epidemiol* 20:307-315). Under no linkage and no association, the resulting allele frequency estimates have the smallest variance among all weighted averages of individual sibship allele frequencies. It is slightly more efficient than the first method if we have sibships of variable sizes. However, given tight linkage, this method may over count alleles for larger sibships and inflate the type I error rate.

Analytical and simulation results show that these methods are more powerful than using just case-control data under a variety of sibship sizes and disease models. Further, when we have data of affected siblings, selection of one affected individuals per sibship in the standard method is quite arbitrary and introduces variability. For example, for an allele of frequency 0.05, it is not unusual to have frequency estimates varying from 0.039 to 0.061 for 200 sib pairs.

**Association analysis by data mining tools.** *P.A. Onkamo*<sup>1</sup>, *P. Sevon*<sup>1,2</sup>, *V.V. Ollikainen*<sup>1</sup>, *H.T.T. Toivonen*<sup>3</sup>, *H. Mannila*<sup>3</sup>, *J. Kere*<sup>1</sup>. 1) Finnish Genome Center, University of Helsinki, Finland; 2) Department of Computer Science, University of Helsinki, Finland; 3) Nokia Research Center, Helsinki.

We have previously developed a data-mining based method, Haplotype Pattern Mining (HPM), to find trait- or disease-associated patterns in densely mapped family or case-control data (AJHG 67: 133-145, 2000). Here, we demonstrate the practical utility of this approach for fast and easy evaluation of association in gene mapping studies. Also, we present a refined method for assessing the ancestral haplotypes and their contribution to the overall association, the TreeDT. The HPM algorithm finds trait-associated haplotype patterns, possibly containing gaps. All patterns found are ordered by their strength of association to the phenotype, and the patterns exceeding a given threshold level are used for prediction of gene location. The list of associated patterns can be used to deduce the number, lengths, and relevance of the ancestral haplotypes to the trait association. The significance of markerwise association scores can be evaluated by a permutation procedure implemented in the program. The method is scalable to any kind of markers, including SNPs. It can be used for populations with different kinds of histories, given that the marker map is dense enough to have marker-marker linkage disequilibrium. The traits may be either binary (categorical) or quantitative. The algorithm is very fast, so, for example, scanning a data of 80 microsatellite markers and 450 chromosomes takes approximately one minute on a Sun Ultra 10 workstation. This also enables high numbers of iterations to be done on permutation tests. The TreeDT is based on discovering and assessing tree-like patterns in genetic marker data similar to the input for HPM. By the TreeDT approach, the historical recombinations, i.e., the coalescent history of a chromosomal area is estimated. The resulting trees are evaluated with a specific tree disequilibrium test. Compared to HPM, TreeDT gives a more detailed picture of the association of specific haplotypes. We have found it to be more powerful than HPM in certain situations, particularly if several founder mutations are present in the data (Sevon et al., in press).

**The use of linkage disequilibrium measures for discriminating between two subpopulations of the North-Netherlands.** *I.M. Nolte, G.J. te Meerman.* Medical Genetics, University of Groningen, Groningen, Netherlands.

Some years ago, Van der Meulen and te Meerman (1997) developed a multi-locus method for measuring linkage disequilibrium between markers. It is based on excess haplotype sharing over the linkage equilibrium expectation. Haplotype sharing was defined as the number of markers that two haplotypes share going from a locus both in telomeric and in centromeric direction. The mean sharing of all pairs of haplotypes is compared with the mean haplotype sharing under linkage equilibrium (LE). The LE expectation is simulated by randomizing the observed alleles over the haplotypes independent of other loci. We tested this linkage disequilibrium measure on both simulated and empirical data. Firstly, haplotypes were simulated according to a Markov chain with a variety of different inter-marker correlations in order to test the validity and the sensitivity of this measure. Secondly, haplotypes were obtained from several projects for which we collected DNA of trios. These trios had to come from the three provinces Friesland, Groningen and Drente in the North-Netherlands. From linguistic studies, it appeared that there are differences between the two subpopulations of Friesland and Groningen. We therefore selected unrelated Friesians and Groningers on the basis of postal codes of their grandparents. This yielded so far 92 Groninger and 763 Friesian haplotypes. LD was determined in the Groninger haplotypes and in 100 subsets of 92 randomly selected Friesian ones. P-values for the tests whether there is more LD in the Friesians were determined by use of randomization statistics. It appeared that indeed the Friesians form a more homogeneous population, as LD in the Friesian haplotypes is significantly stronger than in Groninger haplotypes. In the near future, we hope to compare these results with LD in the Hutterites in order to get an idea of the strength of LD in these two populations. Van der Meulen, M.A. and te Meerman, G.J. 1997. Association and haplotype sharing due to Identity by Descent, with an application to genetic mapping. In Genetic mapping of disease genes (eds. Pawlowitzki, I-H., Edwards, J.H. and Thompson, E.), p 115-135. Academic Press, London.

**SNP analysis of the human chromosome 22 using APEX arrays.** A. Metspalu<sup>1</sup>, J. Zernant<sup>2</sup>, E. Lõhmussaar<sup>1,2</sup>, R. Mägi<sup>2</sup>, K. Kepp<sup>1</sup>, T. Puurand<sup>2</sup>, N. Tõnisson<sup>2</sup>, I. Valvas<sup>2</sup>, H. Pavel<sup>2</sup>, G. Slavin<sup>2</sup>, R. Andreson<sup>1,2</sup>, A. Kurg<sup>1</sup>, M. Remm<sup>1,2</sup>.  
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Sequence variants in the human genome are responsible for the genetic component of disease, individuality and drug response. In order to find associations between SNPs and phenotype, large sample sets need to be genotyped with high-density markers. Large-scale, population-based case-control studies have been limited because neither SNP maps nor genotyping technology have been adequate to fulfill these needs yet. With chromosome 22 fully sequenced and an SNP map constructed, we have performed genotyping on Estonian samples and CEPH families with 1279 SNP markers. An array with 5200 oligonucleotides was spotted to genotype each SNP twice from both DNA strands simultaneously using APEX technology. Allele frequencies, Hardy-Weinberg equilibrium and heterozygosities were calculated for each typed marker. Software was developed to calculate  $D'$  for the whole 33Mb of ch22 and GOLD (*Graphical overview linkage disequilibrium*) plots were constructed. Preliminary results demonstrate that LD is not continuous, and there are islands with high LD separated by low LD spots. Also, the APEX genotyping technology itself was tested by generating over 270 000 genotypes. It appears that SNPs from TSC will need additional testing to find out useful SNPs in respect of the technology and genetics.



**The USH1C gene in Acadians contains at least three rare variants.** *B.J. Keats<sup>1</sup>, S. Savas<sup>1</sup>, B. Frischhertz<sup>2</sup>, M.Z. Pelias<sup>1</sup>, M.A. Batzer<sup>3</sup>, P.L. Deininger<sup>2</sup>.* 1) Department of Genetics, Louisiana State University Health Sciences Center, New Orleans, LA; 2) Tulane Cancer Center, Department of Environmental Health Sciences, New Orleans, LA; 3) Louisiana State University Biological Computation and Visualization Center, Baton Rouge, LA, USA.

Mutations in the USH1C gene, which encodes harmonin, are associated with Usher syndrome type IC (USH1C). In Acadian patients, a mutation (216G>A) was identified in exon 3 and a 45 base pair variable number of tandem repeat (VNTR) polymorphism was found in intron 5 of the USH1C gene. The Acadian patients were found to be homozygous for the 9-repeat allele of this VNTR, which had an unusual structure. PCR-amplification of the VNTR region and restriction enzyme analysis of exon 3 showed that this 9-repeat VNTR is in complete linkage disequilibrium with the 216G>A mutation in exon 3 in the Acadian population. Of 58 unaffected and unrelated Acadians, one was heterozygous for the 9-repeat VNTR allele and the 216G>A mutation, indicating that the disease frequency may be as high as 1 in 14,000 in this population. Analysis of 340 random samples from five ethnic groups (African, African-American, Asian, European, Hispanic) showed the presence of a 9-repeat VNTR in only one Hispanic sample. Thus, the 9-repeat VNTR allele is rare in populations around the world. The Hispanic individual did not have either the 216G>A mutation or the Acadian VNTR structure, suggesting that the 216G>A mutation and the associated 9-repeat Acadian VNTR are restricted to the Acadian population. Additionally, analysis of haplotype data for surrounding markers shows significant linkage disequilibrium over more than six centimorgans, supporting a fairly recent origin of both the 216G>A mutation and the 9-repeat Acadian VNTR in the Acadian population.

**Incorporating parental phenotypes into the quantitative TDT.** *B.S. Maher, M.M. Vanyukov, R.E. Ferrell, M.L. Marazita.* University of Pittsburgh, Pittsburgh, PA.

The transmission disequilibrium test for quantitative traits (QTDT) is a powerful method for analyzing association between genetic markers and quantitative traits. An advantage of the QTDT is that it avoids spurious association due to population stratification. A major disadvantage of QTDT is the need to collect parental genotypes, or sibs to reconstruct parental genotypes. This problem is magnified in several situations (e.g., late age-of-onset). To address these difficulties, several methods have been proposed to eliminate the need for parental genotypes in the family based framework. The focus here is the situation in which parents are readily available and can be easily and inexpensively phenotyped. One potential advantage of collecting parents is the incorporation of parental phenotypes into the QTDT. We explored the power gain from using parental phenotypes as covariates in the QTDT. Specifically, we included parental phenotypes using two approaches. In the first approach, we incorporated both parents phenotypes as covariates in the QTDT as proposed by Rabinowitz (*Hum Hered* 1997; 47: 342-350). In the second, we treated each parental transmission separately while using a trait value equal to the residual of the regression of the offspring phenotype on the opposite (non-transmitting) parent's phenotype. Additionally, we applied the method to parent-child trios collected as part of our ongoing research into the liability to Attention Deficit Hyperactivity Disorder.

**When is the bias caused by population stratification negligible?** *R. Jiang, J. Dong.* Dept Mathematical Sci, Michigan Technological Univ, Houghton, MI.

Population stratification is always a concern in association analysis. It is well documented that case-control samples from non-homogeneous populations could cause bias in association measures. There are three possible remedies. The first one is to match cases and controls. It is difficult to know how much matching between cases and controls is enough. The second is to use family based methods, like the transmission/disequilibrium test. For late onset diseases, collecting family data is difficult. The third is to test the existence of population stratification by using unlinked markers. Since population stratification does not always cause significant bias, we believe that it is better to estimate the bias itself than to test the existence of population stratification. Our goal is to provide a formula for practitioners to estimate the bias caused by population stratification in individual situations. They can decide whether the bias is negligible, and whether further remedies need to be taken. Our method is to consider a homogeneous and a non-homogeneous populations with the same allele frequencies, penetrances, and prevalences. The non-homogeneous population consists of  $k$  subpopulations with different allele frequencies etc. We then find a formula for the ratio of an association measure  $p(\text{excess})$  in homogeneous and non-homogeneous populations. Our conclusions are: The farther the marker locus is from the disease locus, the larger the bias will be. The older the disease mutation is, the larger the bias will be. Thus, the smaller the linkage disequilibrium is, the larger the bias will be. For the purpose of fine mapping, population stratification is not a serious problem if the disease is not too old (less than 400 generations old) and the maximum genetic distance between the marker and disease loci is less than one cM. There is also no need to take population stratification into account if the genetic distance is less than two cM and the disease mutation is less than 200 generations old. In case-control studies, in order to avoid false positives caused by population stratification, it is enough to stratify the sample according to large ethnic groups. It is unnecessary to further stratify Caucasians according to their ethnicity.

**The Use of the Disposition Model For Genetic Analysis. II. Regression of Genetic Markers on Disease Status in Case-Control and Sib-Pair Studies.** *J. Kwagyan, V. Apprey, G.E. Bonney and Statistical Genetics and Bioinformatics Unit. Natl Human Genome Ctr, Howard Univ, Washington, DC.*

Sib-pair analysis is a tool widely used for the genetic determination of complex diseases. Affected sib-pairs should share alleles IBD more often than expected under random Mendelian segregation. In practice, the situation is more complicated because one cannot unambiguously determine the number of alleles shared IBD at every position along the genome. Methods for sib-pair analysis are primarily based on studying individual genetic markers one at a time. When data on controls are available, it is usual to use them only for association studies and to ignore them in linkage analysis. Conversely, in regression analysis to determine the association of markers with a given disease, the dependence among sib-pairs is ignored or a member of one affected pair is chosen. Association and linkage may be mixed in a way difficult to distinguish in practice. Here, we study an application of the disposition model of Bonney 1998, which allows for within-individual and between-sib dependence in the regression analysis of genetic markers on disease status in case-control and sib-pair studies, separately or simultaneously. Within-person dependence measures departures from Hardy-Weinberg equilibrium; between-sibs dependence modeled in terms of IBD measures departures from no linkage; while the regression of the logit of the population allele frequency on disease status and other covariates permits the assessment of marker disease association. A structured model fitting process is introduced allowing these effects to be assessed in turn. The calculations are implemented in the computer package, G.E.Ms.

**Logistic Regression analysis for case/control genetic association studies: combining genetics and epidemiology. F.**

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The ultimate goal of genetic association studies is to identify gene(s) responsible for a given disease. To detect a genetic association the easiest design is the case-control approach, which usually applies a chi-square analysis to look at the significance of an association. The design is simple and does not depend on any population genetic theory or model, other than the one that the strength of an association between genes increases when physical and genetic distances between them decrease. The major problem with the case-control design is the identification of an appropriate control sample to avoid spurious associations due to potential confounding factors related to population admixture or stratification. To deal with these issues, various methods have been proposed, such as the use of unrelated polymorphisms together with the candidate marker(s) to evaluate the extent of "random" association (Devlin & Roeder, 1999). We propose an alternative approach to detect genetic association with a case-control design, based on the application of a logistic regression (LR) model. The LR model allows for the simultaneous consideration of risk and/or qualitative and quantitative confounding factors. The LR outcome is equivalent to the Mantel-Haenszel test (MH) for multiple contingency tables or to other procedures, like the Cornfield test. Moreover, the LR approach has the advantage that allows for multiple confounding factors, which may or may not show a combined or complex interaction effect on the outcome measure. A working example of the LR approach is provided and compared with the simpler M-H and Cornfield statistics, showing that the major advantage of LR concerns the estimate of the Odds Ratios.

**Linkage Disequilibrium in the Mixe Amerindians and Han Chinese across three large genomic regions.** *K.A. Mather, G. Thomson.* Integrative Biology Department, The University of California, Berkeley, CA.

Analysis of a northern European population reveals regions of several cM with a high density of markers in linkage disequilibrium (LD). In ten regions of the genome, this clustered LD is greater than that found in the HLA (Human Leukocyte Antigen) region (Huttley et al., *Genetics* 152:1711-22). The high LD regions identified are likely to be experiencing selection in the Caucasian population, making them good candidates in a search for regions experiencing selection in other populations. However, if selection pressures vary among populations evolving in different environments, patterns of LD may not be consistent between populations. It is therefore of interest to determine if the high LD regions identified in Caucasians also exhibit high LD in other populations with different demographic histories.

We analyzed LD in two non-Caucasian populations (Mixe Amerindians from the Oaxaca valley in Mexico and Han Chinese) for regions on chromosomes 6 (the HLA region), 20 (a control region that did not show high LD in the Caucasian population), and 22 (a region of high LD in the Caucasian population). The Mixe population is small and isolated. In contrast, the Han population has historically been larger, less isolated, and have experienced more population growth. These populations have been separated for relatively long amounts of time, so it is likely that each population has been subject to distinct selective pressures. We examined eleven (CA)<sub>n</sub> microsatellite markers spaced about 0.5 Mb apart in each region for forty individuals from each population. We measured the amount of LD in each region relative to the HLA region. We found that LD patterns are (a) heterogeneous among regions on chromosomes 6, 20 and 22 and (b) different among Caucasian, Han and Mixe populations for a given region. These results show that LD patterns are both population specific and species wide.

This work was supported by NIH grant GM35326 and NSF-DDIG 73054. KAM is an HHMI Predoctoral Fellow.

**Family-based tests of gene-gene and gene-environment interaction for general nuclear family configurations.** *S.L. Lake, N.M. Laird.* Biostatistics, Harvard School of Public Health, Boston, MA.

While assuming that disease susceptibility is principally due to one genetic locus has been used with much success in mapping studies, this assumption may not suffice when exploring the role candidate genes play with other genes and/or exogenous factors in modifying disease risk. In these cases, tests that detect gene-gene and gene-environment interactions are required.

We present a simple test statistic that can be used to study genetic interaction. It is applicable to any type of nuclear family configuration (eg missing parental information and/or multiple (un)affecteds), while still retaining the robust properties of family-based association tests. The method is sensitive to interactions on the multiplicative scale. Statistical inference is provided through a permutation algorithm that produces valid p-values while avoiding tenuous asymptotic assumptions.

The method is illustrated and compared to other approaches via simulation and by application to the family data of the Childhood Asthma Management Program (CAMP). Of interest in the CAMP study is the identification of genes and environmental exposures that may act synergistically in the development of childhood asthma.

**Association analysis of angiotension I-converting enzyme (ACE)-gene polymorphisms with blood pressure. X.**

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The angiotension I-converting enzyme (ACE) gene polymorphisms have been studied intensely in cardiovascular disease (CVD), although the genes contribution to CVD is still being defined. In this study, we sampled 611 and 640 individuals from 188 African-American and 155 White families, respectively. We genotyped 6 polymorphisms within the ACE gene. Single polymorphism association analysis showed marginal association between diastolic blood pressure and one of the polymorphisms in White population ( $p=0.04$ ). Haplotypes were then resolved using the program SIMWALK2 in two populations separately. The cladistic analysis between ACE activity and haplotypes defined two clades which had a significantly differently effect on ACE activity in both populations. Using the two clades, we performed association analysis with BP again. Significant evidence of association to DBP was found for white sample ( $p=0.02$ ), and marginal evidence of association to DBP was found among African Americans ( $p=0.05$ ). This finding of an association between the ACE gene and DBP is consistent with our recent finding in a Nigerian sample. In conclusion, we found that the ACE gene variants associated with ACE activity also affect blood pressure.



**Distribution of Crohn's disease susceptibility haplotypes in European families.** *H. Zouali<sup>1</sup>, S. Lesage<sup>1</sup>, M. Chamaillard<sup>1</sup>, JP. Czarnd<sup>2</sup>, J. Belaiche<sup>3</sup>, S. Almer<sup>4</sup>, C. Tysk<sup>5</sup>, C. O'Morain<sup>6</sup>, M. Gassul<sup>9</sup>, R. Modigliani<sup>7</sup>, JF. Colombel<sup>8</sup>, M. Sahbatou<sup>1</sup>, G. Thomas<sup>1</sup>, JP. Hugot<sup>1</sup>.* 1) Fondation Jean Dausset, Paris, France; 2) Paris, France; 3) Liege, Belgium; 4) Linkping, Sweden; 5) Rebro, Sweden; 6) Dublin, Ireland; 7) Paris, France; 8) Lille, France; 9) Barcelona, Spain.

**Background** : IBD are complex genetic disorders of unknown aetiology. The NOD2 gene was recently identified as a susceptibility gene for Crohns disease. Three NOD2 gene mutations were identified on the same common background.

**Aim** : To estimate the distribution of the 3 CD susceptibility haplotypes in Europe. **Patients and Methods** : A total of 232 CD families recruited through a large European consortium were genotyped for 8 intragenic SNPs located within the NOD2 gene. Haplotypes were build using the GeneHunter package. The 4 haplotypes defined by the same common background were considered. Three of them carrying each of the 3 mutations R675W, G881R or 980fs981X and one haplotype without any of these variants were analysed using one CD patient per family. Haplotypes were classified in 5 groups according to either the geographical origin of the patient ancestors or the recruitment country: France (n=117), Belgium (n=20), Scandinavia (Sweden and Denmark, n=7), SEC (South European Countries including Italy, Spain, Portugal and North of Africa, n=20), and Mixed (patients with ancestors originating from France and an other country, n=16). The frequencies of the 4 haplotypes within the groups were compared by a Chi square test. **Results** : A significant difference was observed in the haplotype distribution ( $\chi^2=21,6$ , ddl=12,  $p<0,05$ ). The haplotype carrying the 980fs981X mutation was more frequent in France and Belgium when compared to the other groups and was not found in Scandinavia. **Conclusion**: The results do not argue for a single founder effect in Europe. Genotyping strategies of the NOD2 gene for genetic diagnosis must be adjusted according to the country of origin.

**Classifying Disease Chromosomes Arising From Multiple Founders.** *K. Yu<sup>1</sup>, R. Martin<sup>1</sup>, J. Meyer<sup>1</sup>, A. Whittmore<sup>2</sup>.*  
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With the increasing availability of high-density marker data, various fine-scale mapping methods have been proposed for using haplotype information to locate disease susceptibility loci. In the setting of the case-control study, samples of case and control haplotypes are collected, and the locus (or loci) for the disease mutation is estimated by comparing haplotype structure among cases to that among controls. One practical difficulty arising in such haplotype mapping is founder heterogeneity; that is, not all case chromosomes inherit a disease-causing mutation from the same ancestral chromosome. One approach to dealing with founder heterogeneity is to separate case haplotypes according to their mutational origins before doing genetic mapping. We propose a non-parametric clustering method to accomplish this goal. The method finds one cluster at a time, with the cluster consisting of case haplotypes that are most likely to share a common founder.

The clustering algorithm has three steps. First, a top-down peeling procedure is used to find a sequence of subsets that are candidates for a cluster. Then, a random permutation procedure is used to find an 'optimal' subset from those candidates. Finally, a bottom-up pasting procedure is used to expand the optimal subset by adding back haplotypes that were mistakenly removed during early stages of the peeling process until a stopping rule is met. Once a cluster has been found, the same procedure can be applied to the remaining set of haplotypes to find other possible clusters. Simulation analyses suggest that in situations with moderately strong ancestral effects (i.e., more than half of the case haplotypes from the same founder), the method can accurately identify the largest group of haplotypes that are descendants from a common founder. Further simulations are being conducted under genetic models more appropriate for complex disorders, based on a study of schizophrenia in a Finnish population.

**Using estimates of individual admixture to study the genetics of phenotypic traits: skin pigmentation in African Americans.** *C.L. Pfaff<sup>1</sup>, E.J. Parra<sup>1</sup>, J. Ye<sup>1</sup>, A. Massac<sup>2</sup>, R.A. Kittles<sup>2</sup>, M.D. Shriver<sup>1</sup>*. 1) Department of Anthropology, Pennsylvania State University; 2) National Human Genomes Center at Howard University.

The availability of molecular panels has been important in the practical application of most genetic mapping methods. Here we report on a panel of ancestry-informative markers (AIMs) that demonstrate high allele frequency differences between Africans and Europeans. These AIMs can be used to estimate the European and African proportional ancestry at the population sample level, subgroup level (e.g. cases and controls for a dichotomous phenotype), and individual level. Estimates at both the subgroup and individual level can be very instructive regarding the genetics of the phenotypes of interest and provide the foundation for mapping the genes underlying these traits (Admixture Mapping). Using skin pigmentation as a model phenotype, we demonstrate how such studies could proceed. We typed 21 AIMs, of which 4 were candidate genes for skin pigmentation differences between Africans and Europeans, in a sample of African American individuals from Washington, DC. The allele frequency difference between Africans and Europeans ranged from 36% to 99.7%. We observed a strong correlation between estimates of individual admixture and skin pigmentation (measured by the melanin index). When analyzed individually, 9 of the AIMs, including 2 candidate genes, showed significant association with skin pigmentation. The high rate of association between AIMs and an ancestry-dependent phenotype, in this case skin pigmentation, indicates that the trait is genetic, and that there is a significant degree of population structure in the sample. In order to correct for this structure, we used the estimate of individual admixture as a covariate. After making the correction, only one candidate gene, oculocutaneous albinism type 2 (OCA2), remained significant, and none of the other AIMs showed association. These results indicate that OCA2 is one of the genes contributing to the differences in pigmentation levels between African and European populations.

**Generating haplotype data with pre-specified pair-wise LD and allele frequencies.** *A. Wille*<sup>1, 2</sup>, *T.F. Wienker*<sup>1</sup>. 1) IMBIE, Bonn University, Bonn; 2) Laboratory of Statistical Genetics, Rockefeller University, New York.

Linkage disequilibrium (LD) mapping using SNPs has proven to be a valuable tool for fine-mapping of disease genes. However, the variation of LD in intensity and range makes it impossible to describe dense sets of SNP markers sufficiently without taking simultaneously into account information on pairwise LD of all marker pairs. Due to its dependencies on marker distance, cross-over interference and grossly reduced informativity of bi-allelic markers, LD between adjacent markers does not determine LD among all marker pairs, although markers are arranged in a linear order on the chromosome.

Correlation among markers influences the performance of fine-mapping methods and simulation studies evaluating this performance should model LD among sets of markers realistically. Here, we explore an approach based on the EM algorithm to generate haplotype data with pre-specified pairwise LD among all markers and pre-specified allele frequencies.

For haplotypes comprising  $k$  bi-allelic loci, the restriction imposed on the data ( $k$  values for allele frequencies and  $k(k-1)/2$  values for the pairwise LD) can be viewed as incomplete data specification that corresponds to multiple complete data specifications of haplotype frequencies. Thus, the EM algorithm provides a mean to determine the maximum likelihood estimate of haplotype frequencies conditional on LD and allele frequency realizations.

The maximum likelihood estimates can then be used directly, or as parameters in an underlying Dirichlet distribution, to generate haplotype data with the pre-specified values of pairwise LD and allele frequencies.

**Strategies for Genome-wide Association Studies: Optimization of Study Designs by Stepwise Focusing Method.** A. Saito, N. Kamatani. Division of Statistical Genetics, Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

**Purpose:** Recently, genome-wide association or linkage disequilibrium (LD) studies have attracted much attention because of the introduction of high-throughput genotyping systems. However, the limitations of such studies are often the total cost for the genotyping or the sample size. Therefore, it is important to estimate the optimal conditions for the study given the limited number of individuals or the limited amount of the cost for the genotyping. In the present study, we have developed "stepwise focusing method" a new approach for such studies and estimated the conditions that give the maximum statistical power.

**Materials and Methods:** Stepwise focusing method consists of two (or more) steps. In the first focusing step, the samples from both case and control groups are genotyped at a certain number of SNP markers (for example 50,000), and the markers that showed significant inter-group differences by the chi-square test using 2x2 allele number table at a relatively high p-value (for example 0.1) are selected. It means that most of the selected markers in the first step are likely to be the false-positive markers. In the second step, the markers selected in the first step are tested using the samples obtained from a different set of case-control samples. In the second step, a larger number of subjects and a lower p-value are used for the selection of the markers. We performed an extensive simulation studies to estimate both the type I error rate and the power of the test using various parameters such as genotype relative risk, disease allele frequency, sample size, and so on.

**Results and Conclusions:** When there are limitations in such conditions as sample size and total number of genotypings, the stepwise focusing method turned out to give the optimal conditions and be more powerful than the conventional methods.

**Estimation of small map distances using chromosome data from an isolated population.** *N.H. Chapman<sup>1</sup>, J. Crumley<sup>3</sup>, T.M. Fujiwara<sup>2,3</sup>, K. Morgan<sup>2,3</sup>, E.A. Thompson<sup>1</sup>.* 1) University of Washington, Seattle, WA; 2) McGill University, Montreal, QC; 3) Research Institute of the McGill University Health Centre, Montreal QC.

Modern sex-averaged genetic maps are based on 8 of the CEPH families, in which 184 meioses are scored. Maps are therefore of limited precision, and the order of some markers cannot be resolved. Misspecification of the marker map leads to bias in multipoint linkage analysis, and accurate fine-scale maps are desirable for linkage disequilibrium mapping. We present a novel approach to the estimation of small map distances, using the pedigree of a large isolated population (North American Hutterites), and simulated genetic data on chromosomes sampled from that population.

A *junction* is a point on a chromosome where DNA from two distinct ancestors meets. Junctions are formed when a crossover event occurs in a region where the two parental chromosomes are not identical by descent relative to the founder population. Therefore the number of junctions accumulated in an interval contains information about the genetic length of the interval. We sample individuals from the population, and count the number of junctions in the interval of interest on each of their chromosomes. Using the pedigree for these individuals, we calculate the expected value, variance and covariance of the number of junctions existing in that interval on each chromosome. These moments depend only on the genetic length  $d$  of the interval. We use a quasi-likelihood approach to model data consisting of junction counts in the interval whose length we wish to estimate, on  $2n$  chromosomes from  $n$  individuals of the population.

Our approach results in unbiased estimates of  $d$ , and good estimates of the variance of the estimate. The standard deviation of the estimate based on a dataset of 200 chromosomes from 100 randomly selected Hutterites is approximately half that for the traditional estimate based on 184 meioses in the CEPH pedigrees. We also describe some of the challenges involved in extending this approach to more realistic data.

**Markov chain Monte Carlo methods for the calculation of likelihoods in genetic linkage studies.** *A.W. George<sup>1</sup>, M. Bogdan<sup>1</sup>, E.M. Wijsman<sup>2</sup>, E.A. Thompson<sup>1</sup>.* 1) Statistics, University of Washington, Seattle, WA; 2) Division of Medical Genetics, University of Washington, Seattle, WA.

Mapping disease genes via linkage analyses requires the calculation of likelihoods on pedigrees which often contain missing marker phenotypes, inbreeding loops and highly polymorphic regions of linked markers. For such data the space of underlying inheritance patterns, upon which exact and sampling based calculations are based, is huge and tightly constrained by observed marker phenotypes. Consequently, exact likelihood computations are intractable and sampling based methods suffer irreducibility and convergence problems. Presented is a sampling based method for the estimation of likelihoods in general pedigrees. This estimation process is less susceptible to mixing problems due to the inclusion of a number of recent advances in Markov chain Monte Carlo methods, namely joint updates of the patterns of inheritance across loci and meioses, integrated proposals, Metropolis-Hastings restarts via sequential imputation and Rao-Blackwellization estimators. The methodology is assessed through its application to three sets of real data originating from a genetic study into Early-Onset Alzheimers disease. Two of the data sets consist of three linked markers inviting comparison with exact results. The third data set demonstrates the true potential of the methodology where a gene is mapped relative to 11 tightly linked markers. Work supported by NIH grant GM-46255.

Program Nr: 1338 from the 2001 ASHG Annual Meeting

**Optimal Study Designs for Detecting and Replicating Susceptibility Loci using Affected Sib-Pair Linkage Analysis.** *M.E. Smolkin, L.W. Hahn, J.L. Haines, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

Minimizing type I errors (i.e. false-positive results) is of primary interest in the linkage analysis of complex multifactorial diseases. Cross-validation (CV) is an approach to minimizing type I errors that has been applied to a variety of linkage studies, most commonly by distributing families into a group used to detect susceptibility loci and an independent group used to verify or replicate the linkage findings. For a single major locus, equal sample sizes provide the most power. However, the percentage of families in each group that is required to maximize power for detecting multiple loci is not currently known. To investigate this question, we simulated a 10cM map in 1200 affected sib pairs. We simulated 100 datasets for each of four different two-locus models (dominant by dominant, dominant by recessive, recessive by recessive, and additive) using previously published disease allele frequencies and penetrance functions (Goldin and Weeks, *AJHG* 53:908-915, 1993). Each functional locus was located on a different chromosome and tightly linked to one marker. Additional complex models are under study. We randomly divided each dataset into a detection group and a replication group ranging in sample size from 100 to 1100 and 1100 to 100, respectively. For each division of the data we assessed the power of affected sib-pair linkage analysis to detect and to replicate each functional locus using an MLOD of 1.0 as the criterion for statistical significance. For the models studied, we found that detection and replication datasets of equal size provided the maximum power to both detect and replicate the susceptibility loci. These results validate that the common approach of using equally sized datasets may in fact be the optimal approach.



**Comparison of age at diagnosis, histologic subtype, and site of tumor in patients with familial vs. sporadic non-Hodgkin lymphoma.** *C.M. Vachon, T.M. Habermann, J.R. Cerhan.* Mayo Clinic, Rochester, MN.

A family history of hematolymphoproliferative malignancies has been associated with an increased risk of non-Hodgkin lymphoma (NHL). However, there are few data on the characteristics of familial versus sporadic NHL. Using the NHL registry at the Mayo Clinic, we conducted a study to compare clinical characteristics (age at diagnosis, histologic subtype, and primary site of NHL) between patients with sporadic and familial NHL. In 2001, we identified all new cases of adult NHL diagnosed between 1986 and 2000 at the Mayo Clinic and mailed out a family history questionnaire to those not known to be deceased. The questionnaire solicited extensive family history information on lymphoma and leukemia in all first-degree ( $1^{\circ}$ ) relatives. Each NHL patient was classified into one of four family history (FH) categories based on their self-report of leukemia or lymphoma in  $1^{\circ}$  relatives: no FH, FH of lymphoma, FH of leukemia, FH of both lymphoma and leukemia. We identified 2038 living cases of NHL diagnosed between 1986 and 2000 for the mail-out questionnaire; of these, we had a current address on 1790. We received complete FH information on 1241 patients (69%). Age at diagnosis of NHL ranged from 17-89 years (mean=65 yrs.) and 54% of our cases were male. First-degree FH of lymphoma was reported by 72 patients (6%),  $1^{\circ}$  FH of leukemia by 50 patients (4%) and  $1^{\circ}$  FH of both in 12 (1%), comprising 167  $1^{\circ}$  relatives with leukemia or lymphoma. FH was most often reported in a brother (35% of time for lymphoma, 39% for leukemia and 50% for both). There was no association between histologic subtype (diffuse, follicular, small lymphocytic, high-grade or peripheral T-cell) or site of NHL (nodal versus extranodal) and  $1^{\circ}$  FH. However, there was an association between age at diagnosis and  $1^{\circ}$  FH (p-value=0.04); patients with no FH had an earlier age at diagnosis than patients with a FH (mean=62.9 for no FH vs. 66.2, 68.3, 64.5 for FH of lymphoma, leukemia and both, respectively). This association has also been seen in a population-based case-control study of NHL and is contrary to evidence from other cancer sites that illustrate earlier ages at onset for genetic forms of cancer.

**Sibling risk ratios and correlations of social and cognitive traits in autistic sib pairs.** *R.M. Cantor-Chiu<sup>1</sup>, M. Alarcon<sup>2</sup>, D. Geschwind<sup>2</sup> and AGRE Consortium.* 1) Human Genetics; 2) Neurology, UCLA, Los Angeles, CA.

Autism is genetically complex with an early childhood onset and a sibling relative risk estimated between 45 and 150. The disorder is characterized by repetitive-restricted behaviors and deficits in language and social skills. Results of genome scans of the autism diagnosis have been inconsistent. Sub-phenotypes of autism displaying a significant sibling risk ratio (srr) or correlation (sc) within autism affected sib-pairs, are good candidates for linkage analysis and can be useful for stratification of families in efforts to reduce heterogeneity. We estimated the srr of categorical items and sc of quantitative items of the Autism Diagnostic Interview (ADI) in 152 affected sibling pairs from The Autism Genetic Resource Exchange (AGRE; Geschwind et al., 2001). The fraction of positive responses to an item (p1) was estimated in the first-born autistic sibs (fbs). P2 is the fraction of positive responses in the second born autistic sibs (sbs) of only positive fbs.  $Srr = p2/p1$ . Items specifically addressing obsessive and compulsive manifestations were tested. While .19 of fbs resisted trivial changes in their environment, .53 of their sbs showed similar resistance ( $srr = 2.76, p = .00001$ ). .50 of fbs and .66 of sbs exhibited a repetitive use of objects ( $srr = 1.33, p = .01$ ). Srr estimates for 1) problems with changes in routine, 2) presence of compulsions and rituals, and 3) unusual preoccupations, were not increased. Special musical talent was seen in .18 of fbs and .33 of their sbs ( $srr = 1.8, p = .04$ ). Quantitative ADI items assessing cognitive development and exhibiting significant sibling correlations were: age at first word ( $r = .32, p = .0006$ ), age at first phrase ( $r = .26, p = .02$ ) and their difference ( $r = .23, p = .05$ ). While these srr estimates are lower than those for the autism diagnosis, fewer genes may contribute to these more specific phenotypes and individual genes may have higher srrs. As with all genetically complex disorders, the effects of common sibling environment, which can be estimated from the study of autistic twins, may be responsible for these significant risk estimates and correlations.

**Attention-Deficit/Hyperactivity Disorder (ADHD): Power simulations to detect linkage in extended pedigrees in the Paisa community of Colombia.** *M. Arcos-Burgos*<sup>1,2</sup>, *F.X. Castellanos*<sup>3</sup>, *F. Lopera*<sup>2</sup>, *D. Pineda*<sup>2</sup>, *L.G. Palacio*<sup>2</sup>, *K. Berg*<sup>1</sup>, *J. Bailey-Wilson*<sup>1</sup>, *M. Muenke*<sup>1</sup>. 1) NHGRI, NIH, Bethesda, MD; 2) University of Antioquia, Medellin, Colombia; 3) NIMH, NIH, Bethesda, MD.

Twin, adoption, family and association studies have shown that genetic factors contribute to the etiology of Attention-Deficit/Hyperactivity Disorder (ADHD). Several of these studies converge in explaining the predisposition to ADHD as the consequence of a Mendelian major gene. Additionally, purely environmental or cultural causes have been excluded. We have collected pedigree data on ADHD from multigenerational families with the goal of genetically mapping a putative gene predisposing to ADHD. All ADHD phenotypes were based on DSM-III and DSM-IV criteria. Here, we present the results of a power simulation using SIMLINK to detect linkage of ADHD to simulated marker loci in these families. ADHD was assumed as a dichotomous trait with incomplete penetrance and a phenocopy rate of 3% in males and 0.2% in females. We simulated cosegregation of the trait and a marker locus in our pedigrees with a set of recombination fractions. The ADHD trait was assumed to be genetically heterogeneous between pedigrees and different proportions of families linked to the same loci (alpha) were assumed. As shown in Table 1, in 27 of the most informative families, a LOD of 27.41 ( $\alpha=100$ ,  $\theta = 0.0$ ) was estimated. Even with an  $\alpha$  value of 25% at a  $\theta$  value of 0.05 enough power to detect linkage is observed for these pedigrees. These data demonstrate the relative benefits of using extended and multigenerational families for genetic mapping studies in ADHD as opposed to using nuclear or sporadic cases. Table 1.

Theta	eLOD 25%	eLOD 50%	eLOD 75%	eLOD 100%
0	2.40	7.45	16.00	27.41
0.05	2.15	5.87	12.08	19.56

**A comprehensive analysis of ch.6p21-22 region in multiple sclerosis (MS).** *L.F. Barcellos<sup>1</sup>, J.L. Haines<sup>2</sup>, J.B. Rimmler<sup>3</sup>, S. Caillier<sup>1</sup>, R.R. Lincoln<sup>1</sup>, M.A. Pericak-Vance<sup>3</sup>, S.L. Hauser<sup>1</sup>, J.R. Oksenberg<sup>1</sup>.* 1) University of California, San Francisco, CA; 2) Vanderbilt University Medical Center, Nashville, TN; 3) Duke University Medical Center, Durham, NC.

An underlying and complex genetic susceptibility plays a clear role in multiple sclerosis (MS) etiology. The HLA-DR2 haplotype (DRB1\*1501 DQB1\*0602) within the major histocompatibility complex (MHC) on chromosome 6p21 has consistently demonstrated both linkage and association with MS in family and case-control studies. Several recent reports of MS and other HLA-associated autoimmune diseases including type 1 insulin dependent diabetes (IDDM) and inflammatory bowel disease (IBD) suggest the presence of a second susceptibility locus telomeric to HLA-DR near microsatellite marker D6S461. In this study, 404 rigorously ascertained MS families were examined (total genotyped individuals=1864) comprised of 98 multiplex (total affecteds=271, ASPs=172) and 306 simplex families. An initial screen of 6p21-22 was performed in multiplex families using 24 markers spanning approx. 20 Mb. Linkage and association (using pedigree disequilibrium test or PDT) to HLA-DR (MLS=3.9, q=0.15; p=0.01 respectively) and nearby markers G51152 and TNFB (MLS=2.9, q=0.20 and 2.5, q=0.20; p=0.02 and 0.01, respectively) was observed. Modest evidence for linkage to D6S461 (MLS=2.4, q=0.20), located 10 Mb telomeric to HLA-DR, was also present. HLA-DR, D6S461 and 3 flanking markers (D6S1691, D6S1050 and D6S1029) were also examined in simplex families. Although a strong DR association was detected ( $p < 10^{-7}$ ), significant results for other markers were not observed in simplex or combined family datasets. The combined dataset was also stratified based on DR2 status in affected members to identify potential interaction effects within the 6p21-22 region; none were detected. Though modest linkage to D6S461 was found in multiplex families, it was restricted to DR2 positive families only, and can be attributed to the close proximity of this marker to DR. Although an additional gene (s) within the MHC may modulate clinical expression in a subset of patients, our findings do not support the presence of a second MS susceptibility locus near D6S461.

**Candidate gene validation and disease gene identification in a population from Northern Sweden.** *M. Holmberg, J. Jonasson, K. Lejon.* UmanGenomics, Umea, Sweden.

The molecular tools required to dissect complex disease are becoming available. Discovery genetics, expression analyses and animal models now generate large numbers of candidate genes that may predispose to or cause complex disease. The next critical step is to associate variations in the candidate genes with the appropriate disease phenotype. The essential factors in association studies of complex disease is the population structure, the number of well defined cases available for analysis, and last but not least the quality of the controls. The powerful approach of using unrelated cases and controls is limited by the ability to find adequately matched controls. The optimal controls can be identified in a prospective population-based cohort where there is no bias in the selection of cases and controls. UmanGenomics has access to a 15 years old prospective population-based Biobank, collected from the relatively homogenous population of Vasterbotten in Northern Sweden. The Biobank contains DNA and Plasma samples and biochemical and life style data from >66.000 individuals. The Biobank is integrated with high quality disease registers, and today a substantial number of well characterized cases of common diseases like diabetes, myocardial infarction, stroke and prostate cancer are available. Cases can be matched with controls collected under identical conditions with identical biochemical and environmental information available, which ensure that optimal controls are selected - a prerequisite for success in association studies of complex disease. Furthermore, a number of subphenotypes of disease including glucose tolerance, blood lipid levels, blood pressure etc. has already been determined for all 66.000 individuals and other biochemical markers can be analysed in the plasma fraction. This allows for the association of less genetically complex phenotypes to genetic variation. UmanGenomics resources are ideally suited for association studies of complex disease. We will in detail describe the advantage of our Biobank for candidate gene validation and present our program for disease gene identification.

**Testing for population subdivision in four large case-control cohorts.** *K.L. Lunetta<sup>1</sup>, R.S. Wells<sup>1</sup>, M. Seielstad<sup>2</sup>, K. Ardlie<sup>1</sup>*. 1) Genomics Collaborative, Inc, Cambridge, MA; 2) Harvard School of Public Health.

Population subdivision is often implicated as a cause of the high number of false-positive disease-marker associations reported in the literature, but to date few if any carefully matched case - population control samples have been rigorously examined to determine if detectable population subdivision is present. Here, we investigate population subdivision in four large case-control samples. A total of 3486 individuals were genotyped for 9 unlinked microsatellite markers chosen to have maximal allele frequency differences among populations. 40 additional random unlinked SNPs are currently also being genotyped. The patient samples consist of 1) 500 US Caucasians with hypertension 2) 500 US Caucasians NIDDM patients 3) 500 Polish NIDDM patients, and 4) 243 US African Americans with hypertension. For all samples, controls were matched 1:1 to individual cases on age, sex, self-reported ethnicity, and, in the US samples, geographic region in which they reside. In the African American sample, we required both parents and all four grandparents of cases and controls to be self-reported African American. In each sample, we tested for substructure using the sum of the individual Pearson  $\chi^2$  statistics comparing case and control allele frequencies for the nine microsatellite markers. We found no evidence for stratification in any of the samples: p-values for the four samples were .63, .34, .48, and .97, respectively. While these markers should have reasonable power to detect relatively obvious substructure, they are unlikely to detect more subtle stratification. The additional 40 SNPs typed for the same individuals will improve our power to detect lower levels of stratification, and will provide a comparison with the microsatellite results.

Our results suggest that carefully matched case-population control samples similar to our US Caucasian, African American, and Polish samples are unlikely to contain gross levels of substructure that would result in large numbers of false positive associations.

**A comparison of cost-effectiveness between microsatellite and single nucleotide polymorphism markers in genomewide linkage disequilibrium testing.** *J. Ohashi, K. Tokunaga.* Dept Human Gen, Grad Sch Med, Univ Tokyo, Tokyo, Japan.

Microsatellite (MS) markers are expected to be more powerful for linkage disequilibrium (LD) testing, compared to single nucleotide polymorphism (SNP) markers. However, more SNPs can be analyzed at the same cost because of the low-cost genotyping. Although the efficiency in LD testing has been compared between MS and SNP markers, the efficiency should be determined by a cost-effectiveness, or resources of money in the study. In order to address this problem, a simple deterministic model is used for the calculation of LD between marker and disease alleles. Here, mutation at the marker locus and recombination between the marker and disease loci are assumed in the model. For the comparison of MS and SNP markers, a statistical power with a proper significance level of  $\alpha$ , corrected by the number of testing, is calculated based on chi-square statistic. The obtained power is largely dependent on the initial situation (e.g., allele frequencies of the disease and marker loci). Also, the power is influenced by the number of testing when the Bonferroni correction is applied. As the number of markers is increased, the genetic distance between the disease and the marker loci is decreased. Although LD between them becomes strong with decreasing the genetic distance, the power is not always increased due to the Bonferroni correction. The present model will provide a guideline for selecting a suitable genetic marker in genomewide LD testing when the resources of money are limited.

**Genomic Control Methods to Adjust for Population Stratification for Candidate-Gene Association Studies.** *S.L. Slager, D.J. Schaid.* Mayo Clinic, Rochester, MN.

Large-scale case-control studies will be required to find genes of modest effect. However, since unrelated controls may be used, these studies may be affected by population stratification and therefore be subject to an increased false positive rate. To correct for the effect of population stratification, some adjustment to the candidate-gene test statistic ( $T$ ) is necessary. Assuming the candidate gene has two alleles, Devlin and Roeder (*Biometrics* 55:997, 1999) proposed a simple adjustment,  $T/l$ , which asymptotically follows a  $\chi^2$  distribution with 1 df. The quantity  $l$  is estimated from a distribution of  $m$  independent statistics,  $Y_i$ , for  $i=1, \dots, m$ , where  $Y_i$  is generated by testing the association between the trait and the  $i$ th diallelic "null" marker. These  $m$  null markers are assumed to be independent of the disease of interest and of each other. If there is no population stratification effect, the expected value of  $l$  is 1; otherwise it is greater than 1. Devlin and Roeder's proposed method and the method proposed by Reich and Goldstein (*Genet Epi* 20:4, 2001) estimate  $l$  using only the first moment of the marker distribution, but an improved correction can be obtained by using both the first and second moments. A precise correction is important because it can affect the false positive rate or power of the candidate-gene test statistic (for example, a correction that is too large results in a candidate-gene test that is too conservative). We present new methods to estimate  $l$ , as well as new methods to better approximate the distribution of  $T/l$ . The power and the type I error rate of each adjusted candidate-gene test — using the proposed methods — are computed from simulated data and compared with those that were previously proposed by Devlin and Roeder (1999) and Reich and Goldstein (2001).



**A comparison of the power of the variance components method and revised Haseman-Elston method in the presence and absence of non-random ascertainment.** *Y. Yao*<sup>1</sup>, *A. Sorant*<sup>2</sup>, *A. Wilson*<sup>2</sup>. 1) Epidemiology Department, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD; 2) NIH/NHGRI, Baltimore MD.

Although the variance components (VC) method has been suggested as one of the more powerful methods to detect linkage when pedigrees are randomly selected, the power is likely to be reduced when the assumption of a multivariate normal distribution of the underlying trait locus is violated, as under non-random ascertainment. The revised Haseman-Elston (RHE) method, which regresses the cross product of the siblings mean-corrected phenotypes on the proportion of alleles shared IBD, is reported to provide greater power than the traditional H-E method without inflating type I error rate. In this study, the statistical properties of both methods were evaluated, and the power to detect linkage and the type I error rate of the VC and RHE methods were compared under random and non-random ascertainment. In addition to random sampling, we selected families with three ascertainment methods: selecting families with at least one offspring with phenotype exceeding a given threshold (H1); at least two offspring whose phenotypes exceed the threshold (H2); and extremely discordant offspring, one whose phenotype exceeds a high threshold and one whose phenotype is below a low threshold (ED). Results show that the VC and RHE methods have similar power to detect linkage under random ascertainment. For the models considered, the power of the VC method was often increased under ascertainment method H1, but consistently reduced under methods H2 and ED. The power of the RHE method was consistently increased under H1 and ED, but slightly reduced under H2. This comparison can potentially serve as a guideline for choosing statistical approaches in mapping quantitative traits.

**Little Evidence for Bias Due to Population Stratification in a Population-Based Study of Non-Hispanic Blacks and Whites.** *J.S. Pankow<sup>1</sup>, M.A. Province<sup>2</sup>, S.C. Hunt<sup>3</sup>, D.K. Arnett<sup>1</sup>*. 1) Division of Epidemiology, University of Minnesota, Minneapolis, MN; 2) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; 3) Cardiovascular Genetics, University of Utah, Salt Lake City, UT.

Undetected population stratification may increase the probability of finding spurious associations in genetic studies of unrelated individuals, but the magnitude and relative importance of this bias is unclear. We conducted association tests for 6 common phenotypes (obesity, hypercholesterolemia, hypertension, diabetes, renal dysfunction, and cardiovascular disease (coronary heart disease or stroke)) using 368 highly polymorphic markers typed for a genome-wide scan. The study population included 444 unrelated subjects (231 non-Hispanic blacks, 213 non-Hispanic whites) randomly selected from 5 U.S. communities as part of the Hypertension Genetic Epidemiology Network (HyperGEN) of the NHLBI Family Blood Pressure Program. Phenotype prevalences ranged from 9% for renal dysfunction to 50% for hypertension, while prevalence ratios comparing blacks versus whites ranged from 2.4 for renal dysfunction to 0.7 for hypercholesterolemia. In crude analyses pooling both ethnic groups, the percentage of markers statistically significantly associated with diabetes (8.4%), hypertension (7.9%), and renal dysfunction (7.6%) was somewhat higher than that expected under the null hypothesis (5.0%). However, the percentage of associated markers did not exceed 6% for any of the phenotypes after controlling for self-reported ethnicity. Summary tests for population stratification (Pritchard and Rosenberg, 1999) incorporating all 368 markers were statistically significant for diabetes, hypertension, and renal dysfunction in crude analyses, but not after adjustment for ethnicity. These data provide little or no evidence that undetected stratification within self-reported ethnic groups led to an increased probability of finding spurious associations in our study population. Other factors such as gene-environment interactions, linkage disequilibrium, and chance may be more likely explanations for inconsistencies between genetic association studies.

**Comparison of the distributions of FMR1 alleles in a normal population and in a population with cognitive disorders.** *A.H. Alegria, L. Rengifo, H. Serrano, D. Gaviria, E. Aguilar.* Basic Sci, Fac Med, Univ Tecnologica de Pereira, Pereria, Risara, Pereira, Colombia.

The Fragile-X syndrome is one of the most frequent causes of mental retardation in humans and is associated with a fragile chromosomal site at Xq27.3. This fragility is due to the expansion of a repetition 5-(CGG)  $n-3$  that occurs naturally in the 5 UTR region of FMR1 gene. The distribution of this repeat is between 6 and 60 for normal individuals, between 60 and 200 for premutated alleles and more that 200 for affected individuals. A number between 41 and 60 is considered intermediate. We studied the distribution of normal, intermediate and premutated alleles for patients with cognitive and behavior disorders, and for healthy individuals in Risaralda Colombia SA, using PCR and Southern blot techniques. The population with behavior disorders corresponds to cases of autism, autistic behavior, mental retardation excluding Fragile X, learning disorders and attention disorders. We compared the results in the two populations, using statistical analysis. For the normal population, 1171 alleles corresponding to 364 women and 443 men were considered, and for the population with behavior disorders the sample contained 284 alleles of 80 women and 124 men. The proportions of normal, intermediate and premutated individuals in the sample of the normal population were 97.8%, 1.9% and 0.3% respectively. In the population with behavior disorders, the percentages of normal, intermediate and premutated alleles were 97.5%, 1.4% and 0.35% respectively. The most frequent case was 30 repetitions in both groups, with 79.6% of the cases in the normal population, and with 72.44% of the cases in the population with behavior disorders. A test of difference of variances was made, and it was found that the difference is not statistically significant. Given equal variances, the means were compared and it was determined again that there was no significant difference. Finally the proportions of normal, intermediate and premutated individuals were compared in both populations and it was found that they are statistically equal.

**Associations between putative risk factors and Alzheimers disease according to gene polymorphisms using a large case control study.** *Z. Yamagata<sup>1</sup>, S. Tang<sup>1</sup>, Y. Shindo<sup>1</sup>, Y. Takeda<sup>1</sup>, T. Mizutani<sup>1</sup>, T. Asada<sup>2</sup>.* 1) Department of Health Sciences, Yamanashi Medical University, Yamanashi, Japan; 2) Department of Psychiatry, Tsukuba University, Japan.

We evaluated the association between putative risk factors and Alzheimers disease (AD) according to gene polymorphisms. The subjects were 407 patients (168 men and 239 women: age at onset  $69\pm 10$ ) with a diagnosis of probable AD based on the NINCDS-ADRDA criteria and 398 spouses (162 men and 236 women: age  $69\pm 9$ ) of the subjects served as a control. The risk factor interview was developed specifically for this study to assess 17 putative risk factors. APOE gene polymorphisms were determined by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The triplet (CGG) repeat polymorphisms of very low-density lipoprotein receptor genes (VLDLr) were genotyped by the PCR fragment analysis software of Long Read Tower<sup>TM</sup> sequencer. The estimating odds ratio and the multiple logistic analysis were performed by SAS statistic analysis package to evaluate the association. This study was conducted after obtaining written informed consent from the subjects or their guardians. The genetic analysis revealed that the AD was associated with VLDLr as well as APOE: the odds ratio was 2.98 for the e4 allele. The frequency of the 5-repeat allele of VLDLr was significantly higher in AD than in the control ( $p < 0.04$ ). The odds ratio for 5-repeat allele was 1.56 (95% CI=1.32-1.78). Some of the putative risk factors including aging, family history of AD, education years, napping and the death of a partner were confirmed as risk factors for AD. Though most of these risk factors were not interactive with gene polymorphisms, onset age was associated with APOE but not VLDLr. In addition, limited habitual napping for up to 60 min had a protective effect against the development of AD especially for carriers of the APOE e4 (OR=0.49, 95% CI=0.33-0.73).

**A Comparison of Two Methods for Detecting Interactions Among Genetic Polymorphisms in Matched Case-Control Studies.** *C.S. Coffey<sup>1</sup>, P.R. Hebert<sup>2</sup>, J.H. Moore<sup>3</sup>, M.D. Ritchie<sup>3</sup>, H.M. Krumholz<sup>2</sup>, T.M. Morgan<sup>2</sup>, J.M. Gaziano<sup>4</sup>, P.M. Ridker<sup>4</sup>.* 1) UAB School of Public Health, Birmingham, AL; 2) Yale Univ School of Medicine, New Haven, CT; 3) Vanderbilt Univ School of Medicine, Nashville, TN; 4) Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

A matched case-control study is often used to investigate associations between genetic polymorphisms and risk of disease. Traditionally, when many polymorphisms are of simultaneous interest, conditional logistic regression models have been used to examine interactions and estimate appropriate relative risks. However, it becomes difficult to fit a model and examine interactions if the number of polymorphisms of interest becomes moderately large. The Multifactor Dimensionality Reduction Method (Ritchie et al. *AJHG* 69, 2001) eliminates this high dimensionality problem, allowing simultaneous examination of a large number of genetic polymorphisms. However, it is not possible to estimate relative risks using this method if more than two groups are required to adequately summarize the data.

Researchers must weigh the strengths and weaknesses of the two approaches when choosing an analysis plan for a particular study. In this presentation, we illustrate how the two methods may be combined to give a clearer overall picture than that obtained using either method alone. We illustrate the use of the two methods with an analysis from the Physicians Health Study. The study was conducted to examine possible interactions among the angiotensin converting enzyme (ACE) insertion/deletion, plasminogen activator inhibitor-1 (PAI-1) 4G/5G, and tissue plasminogen activator (tPA) insertion/deletion gene polymorphisms on risk of myocardial infarction. The overall results for the two methods were consistent, with both methods suggesting an interaction between the ACE I/D and PAI-1 4G/5G polymorphisms on the risk of MI.

**Non-Parametric Alternatives For Mapping Quantitative Trait Loci Using Genome-Wide Scan Data: Statistical Comparisons and an Application to Slow Beta EEG Waves.** *S. Ghosh*<sup>1</sup>, *B. Porjesz*<sup>2</sup>, *H. Begleiter*<sup>2</sup>, *T. Reich*<sup>1</sup>. 1) Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO; 2) Dept Psychiatry, State Univ New York Health Science Center, Brooklyn, New York.

Non-parametric linkage analysis is more robust than parametric analysis because of its insensitivity of trait distributional assumptions. We have explored two non-parametric methods, one based on ranks (Randles and Wolfe 1979), and the other based on kernel smoothing [KS] (Silverman 1986) for mapping quantitative trait loci using genome-wide scan data on sib-pairs. Our statistical procedure exploits the empirical relationship between the squared difference in sib-pair trait values and the estimated multipoint identity-by-descent scores at different points on the genome. We have developed suitable test statistics for linkage corresponding to the two methods. We have compared these methods with the linear regression [LR] method of Elston et al. (2000) using Monte-Carlo simulations and have shown that our methods are more robust with respect to assumptions underlying trait genotype-specific expectations, variances and epistasis. When there are deviations from the assumptions, the KS method outperforms the LR method. However, as expected, when the assumptions are satisfied, the LR method is more powerful, but only marginally, in detecting linkage than the KS method. Although the rank-based method does not yield high power, it is more powerful than the LR method when there are deviations from distributional assumptions. We have used the KS method to analyze data on Slow Beta EEG waves collected by the Collaborative Study on the Genetics of Alcoholism project. We have obtained statistically significant signals of linkage (evaluated using bootstrap) on Chromosomes 1, 4, 5 and 15. Although many of the linkage peaks are also obtained by the linear regression strategy, the p-values corresponding to the peaks are lower for the KS method, indicating a greater sensitivity of the KS method. We have also investigated the presence of epistatic interactions between regions exhibiting significant linkage. Evidence of epistasis was found between regions on Chromosomes 1 and 4 with those on Chromosome 15.

**A simulation-based method for applying standard statistical tests to extended pedigree data.** *N.J. Camp*<sup>1,2</sup>, *J.M. Farnham*<sup>2</sup>. 1) Genetic Research, IHC, UT; 2) Genetic Epidemiology, University of Utah, UT.

Standard statistical tests such as contingency table analysis, Armitage's trend test, McNemar's test (TDT), regression analysis (quantitative TDT) and survival analysis are valid only when individuals used in the analyses are independent. If biologically related individuals are used, the variance estimates for these statistics are biased and can lead to an increase in type I error. Large extended pedigree resources, ascertained primarily for prior linkage analysis, contain affected individuals with high likelihood of being genetic in nature. Unfortunately, their relatedness invalidates tests such as those mentioned above. Validity can be regained by randomly sampling a single case from each pedigree, but this leads to severe loss of informativeness and power. Extensions to some of the methods listed above, usually for specific pedigree types, have been proposed (Abecasis et al, 2000, George et al, 2000, Martin et al, 2000, Slager and Schaid, 2001). Here we propose a simulation-based approach which is general for both resource structure (mixtures of singletons to large extended pedigrees can be used) and statistical test. The initial step is to select the cases and controls to be used in the chosen test. This can range from a few from each pedigree to all possible. The statistic of interest is then computed for the observed (real) data, for example, a chi-squared statistic for a case-control or TDT, or a t-statistic for a coefficient in a regression. To assess the significance of this observed statistic, an empirical null is simulated. Estimates for allele frequencies (genotype frequencies) are calculated from the total resource, and these values are used to perform a gene-drop through the pedigrees. The resulting genotypes constitute a null genotypic configuration and these are used to calculate a null statistic. Repeating this several hundred times, an empirical null distribution is created and the significance of the observed statistic can be evaluated.

Our method is intuitively simple to understand, flexible in the types of tests to which it can be applied and allows for maximal use of the information in an extended pedigree resource.

**Estimation and Comparison of Ordered Allele Frequencies from Case-Control and Sib-Pair Data.** *G.E. Bonney, V. Apprey, J. Kwagyan.* National Human Genome Ctr, Howard Univ, Washington, DC.

We present results from a systematic study of the estimation of genetic marker allele frequencies when the samples are non-random as in case-control and sib-pair studies, and when the alleles may be ordered by their sizes. Association studies, non-parametric linkage analysis and recent molecular techniques allowing for the measurement of a large number of alleles at each marker locus have created a need to examine the estimation of gene frequencies from such non-random samples. An implication of Hardy-Weinberg equilibrium, commonly assumed in classical and recent approaches, is the random association of alleles in the gene pool during the formation of genotypes. Case-control, sib-pair and other non-randomly selected samples of individuals are not generally representative of the gene pool. In our work, we start with a theoretical definition of population allele frequency that is actually implicit in classical formulations. The definition immediately points to the natural, unbiased and efficient estimator and its standard error, both with explicit formulas. Samples of individuals or well-characterized family units such as sib-pairs may be used. An assumption of Hardy-Weinberg equilibrium is not required. The dependence within such units is automatically taken into account. Another problem created by modern molecular analysis is the large number of alleles ordered by size measured at each locus, which generates very sparse data. We use our formulas to create charts from the empirical distribution function. The chart shows which alleles ordered by size that may be effectively combined. This way of combining alleles is less ad hoc than existing methods. The calculations have been programmed in G.E.MS. Some data on chromosome 20 markers and type 2 diabetes are used as illustration.



**Lack of evidence for familial aggregation of breast and prostate cancer in the Minnesota Breast Cancer Family Study using a correlated frailty model approach.** *D.M. Grabrick, T.M. Therneau, J.R. Cerhan, C.A. Janney, R.A. Vierkant, T.A. Sellers.* Health Sci Research, Mayo Clinic, Rochester, MN.

Evidence for familial clustering of breast and prostate cancer is mixed. We conducted a multisample cohort study of prostate cancer within an existing study of 426 multigenerational families ascertained through female probands diagnosed with breast cancer between 1944 and 1952. Three groups of males were sampled: 804 blood relatives and 536 marry-ins from families with four or more breast or ovarian cancers in the female blood relatives (high risk families), and 484 blood relatives in families where only the proband was affected with breast cancer (low risk). A total of 118 prostate cancers were reported.

To make the most use of the detailed three-generation pedigree data, a Cox model with random effects (frailty) was fit, using the kinship matrix as the correlation structure of the per-subject random effects. This approach fully accounts for the effects of differential follow-up time and age of onset, while accounting for pedigree structure. As expected, fits to only the female/breast or male/prostate data showed a significant genetic component for the risk of incident disease. However, they were not related. The relative risk of prostate cancer associated with the frailty term (which estimates a man's level of genetic risk that is manifest as increased risk of breast cancer in his female relatives) was 1.1 (95% C.I.: 0.8-1.3) with the inclusion of parity in the breast cancer model. Therefore, we did not see any evidence for an association between breast and prostate cancer using this model.

Using ordinary Cox regression, the rate of prostate cancer among blood relatives in high risk families was significantly lower than among marry-ins (RR=0.6, 95% C.I.: 0.4-0.9). The rate of prostate cancer among blood relatives in low risk families was not significantly different from the rate among marry-ins (RR=0.8, 95% C.I.: 0.5-1.2). The fact that a more careful approach did not validate the simple group comparison emphasizes the importance of the methodological approach to studying familial aggregation.

**The influence of zygosity and chorion type on fat distribution in young adult twins — Consequences for genetic twin methodology.** *R.J.F. Loos<sup>1,2</sup>, G. Beunen<sup>2</sup>, R. Fagard<sup>3</sup>, C. Derom<sup>1</sup>, R. Vlietinck<sup>1</sup>*. 1) Centre for Human Genetics; 2) Department of Sport and Movement Sciences; 3) Hypertension and Cardiovascular Rehabilitation Unit, Katholieke Universiteit Leuven, Leuven, Belgium.

The twin design is frequently applied to estimate the relative contribution of genes and environment to a variety of traits, including body composition. An adverse intra-uterine environment has been associated with abdominal fat distribution. Twins often have a low birth weight and a short gestation. Therefore, they may have an increased risk to develop abdominal obesity. Furthermore, monozygotic monochorionic twins (MZMC) have a larger intra-pair birth weight difference compared to monozygotic dichorionic twins (MZDC). If adult anthropometry is programmed in utero, this may affect the intra-pair correlations in adulthood and, consequently, also the results from the classic twin method to estimate genetic and environmental influences. In the present study, we compared the absolute values, the intra-pair differences, and the intra-pair correlations of body mass, height, BMI, and abdominal fat distribution of 424 MZMC, MZDC and dizygotic (DZ) twin pairs (aged 18—34 yrs). DZ, MZDC and MZMC twins did not differ for most anthropometric characteristics. Only MZ women tended ( $p=0.03$ ) to accumulate more abdominal fat compared to DZ twins. Overall, the contribution of zygosity and chorion type to adult anthropometry was rather low (1.7%). Although the intra-pair birth weight difference of MZMC pairs (10.5% in men, 12.3% in women) was significantly larger compared to that of MZDC (6.9% and 9.2% resp.) pairs, the intra-pair differences in adult anthropometry were similar for both MZ twin types. Also the intra-pair correlations of MZMC and MZDC pairs were strikingly alike, suggesting no significant influence of the prenatal environment on adult concordance. In conclusion, the substantial difference in the prenatal environment of MZMC and MZDC twins did not result in a difference in intra-pair concordance of adult anthropometry and fat distribution. Therefore, we suggest that the chorion type of MZ twins does not bias the twin design and the estimation of the genetic contribution to adult anthropometry.

**Relationship inference from trios of individuals in the presence of typing error.** *S. Sieberts*<sup>1</sup>, *E.M. Wijsman*<sup>2</sup>, *E.A. Thompson*<sup>1,2</sup>. 1) Dept Statistics, Univ Washington, Seattle, WA; 2) Dept Biostatistics, Univ Washington, Seattle, WA.

Errors in genetic data consist of two different types: relationship misspecification and genotyping error. Both of these error types can seriously affect the inference drawn from linkage studies. Typical methods for resolving these errors consider pairs of individuals. When possible, considering a third related individual can increase the power to detect both types of error. We have developed a program, using a hidden Markov model, to compute likelihoods, given genotypes at multiple linked loci for trios of individuals, in the presence of genotyping error. This program, ECLIPSE, is available for download from <http://www.stat.washington.edu/thompson/Genepi/Eclipse.shtml>. Here we apply the method to marker data of the well-analyzed COGA study, and to an uncleaned dataset (LPPG). For the COGA data, we find that inferring relationships among trios of individuals requires fewer markers than inferring pairwise relationships. In one example, a pair of reported full sibs has a LOD (base 10) of 0.745 using all 285 markers. When we include another reported full sib in our analysis, the LOD is 14.88. In sibships where the inferred pairwise relationships are incompatible, examining individuals in trios can clarify relationships. In previously uncleaned datasets, allowing for typing error is essential when comparing trios of individuals since a true relationship of full sibs may be excluded by the reported data. In analyses of the LPPG data, we find that allowing for genotyping error can distinguish between full sibs with Mendelian error and more distant relationships. Analysis of trios has the advantage that, even in the absence of parental data, typing errors that cause Mendelian incompatibilities are detected. In one example, we discover a mother-daughter sample switch by including the mother's two siblings. An analysis of the reported sibs indicates 42 Mendelian incompatibilities and a LOD of -29.2, assuming a 2% genotyping error rate. An analysis of the two sibs with the putative daughter result in a LOD of 56.0 in favor of a full sib trio relationship, assuming a 2% error rate. In this inferred sib trio, typing error results in one Mendelian incompatibility.

**Latent class models for identifying patterns of DNA methylation.** *K.D. Siegmund.* Dept Preventive Medicine, Univ Southern California, Los Angeles, CA.

DNA methylation is being measured at 10 candidate genes on a population-based sample of 300 subjects with colorectal adenomas. Investigators propose to identify a subset of patients with aberrant DNA methylation and characterize these subjects with respect to risk factors for adenomas.

In a simulation study, we compare four methods for clustering subjects into groups, based on their pattern of DNA methylation: 1. a methylation index, 2. agglomerative hierarchical cluster analysis (using Manhattan distance and group average linkage), 3. latent class mixture model and 4. latent class classification model. We model dichotomous measures of DNA methylation, as a function of covariates, using the logistic link. For approaches 1 and 2, we cluster both the raw observations and the residuals from a logistic regression analysis. For approaches 3 and 4, we model the covariate effects in the latent class analysis. In the latter two approaches, parameter estimates are obtained using the EM algorithm (mixture model) or CEM algorithm (classification model). We compare misclassification rates and parameter estimates for two unobserved classes, with either no measured covariate effects, locus-specific covariate effects, or subject-specific covariate effects (e.g. age). Overall, we observe the lowest misclassification rate for the latent class mixture model. In a model with age effects, clustering the residuals decreased the misclassification rate for the methylation index for clusters of equal size, but not for clusters of unequal size. In general, clustering residuals increased the misclassification rate for the hierarchical cluster analysis. Parameter estimates for the mixture model appeared unbiased. The classification model appeared to overestimate the difference between group means.

In summary, a latent class analysis is a flexible approach to cluster analysis that permits the incorporation of measured covariates. It can yield lower misclassification rates than alternate approaches, at the cost of more computing time. Currently we are extending the latent class model for measures that are a mixture of discrete and continuous values.

**GenLib: A web-site for power computations for linkage analysis.** *A.A. Todorov<sup>1</sup>, B.J. Keats<sup>2</sup>, A.C. Heath<sup>1</sup>*. 1) Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO; 2) Dept Biometry & Genetics, Louisiana State University Med Ctr, New Orleans, LA.

**Description:** GenLib is a web-accessible software library with a user-friendly interface to perform power computations for twin, linkage and association studies. The aim of the project is to permit power computations under realistic conditions while minimizing the burden to the user. Users can perform power computations under one- and two-locus genetic models, while varying sampling and/or experimental scheme. Specific programs include: searches for efficient sampling schemes for linkage analysis, basic design of experiments in pharmacogenetics, power computations for multipoint linkage analysis with reduced marker polymorphism with user-provided genetic maps and allelic frequencies. The web-site also permits power computations for key ancillary analyses, e.g., latent class/latent trait methods for phenotype definition.

**Output:** Analyses run on our network of Alpha and Sun computers. Output for short analyses is received immediately on screen. For longer analyses, users are directed by e-mail to an URL containing the results of the analyses. Typically, output consists of a short paragraph describing the statistical methods with references, accompanied by summary tables and graphs. All elements, including tables and graphs, can be directly copied into a word-processing program (e.g., Ms-Word or WordPerfect).

**Illustrations:** In the present, we will provide two illustrations of power computations performed using this software: (i) a joint linkage analysis of a dichotomous phenotype and a correlated continuous trait under selective sampling with additional twin data, and (ii) linkage analysis for a multivariate dichotomous phenotypes under non-normal assumptions.

Supported in part by grants AA-12232 and AA-07728.

**Genetic Susceptibility/Resistance to Dental Fluorosis Among Different Mouse Strains.** *E.T. Everett<sup>1</sup>, M. McHenry<sup>1</sup>, N. Reynolds<sup>1</sup>, H. Eggertsson<sup>2</sup>, J. Sullivan<sup>2</sup>, C. Kantmann<sup>2</sup>, A. Martinez-Mier<sup>2</sup>, G.K. Stookey<sup>2</sup>.* 1) Oral Facial Development, Indiana Univ Sch Dentistry, Indianapolis, IN; 2) Oral Health Research Institute, Indiana Univ Sch Dentistry, Indianapolis, IN.

Fluoridation of community water supplies for the prevention of dental caries remains one of the top ten public health interventions of the last century. The safety and efficacy of fluoride use has been well demonstrated. Concurrent with the decline in dental caries has been an increase in the prevalence of dental fluorosis (DF). Between 7.7% to 80.9% of the population in communities with fluoridated water and 2.9% and 42% of the population in communities with nonfluoridated water develop DF. While the increase in DF primarily involves very mild and moderate forms, an increase in the number moderate and severe DF occurs as well. Greater than optimal fluoride exposure comes from a variety of sources: toothpastes and mouth rinses containing fluoride, dietary fluoride supplements, self-applied fluoride gels, and professionally applied products. We hypothesize that DF represents a complex condition caused by both environmental and genetic factors. We have chosen to test this hypothesis using genetically separate inbred strains of mice and because mice continually erupt their incisors (active amelogenesis). Twelve strains of mice (129P3/J, A/J, BALB/cJ, C3H/HeJ, C57BL/6J, C57BL/10J, CBA/J, DBA1/J, DBA/2J, FVB/NJ, SJL/J, and SWR/J) were treated with varying concentrations (0, 25, and 50ppm) of fluoride in their drinking water for 60 days. Utilizing clinical criteria and quantitative light fluorescence (QLF) assessment of lower incisors, mice clustered into three phenotypic groups: resistant strains (129P3/J, FVB/NJ, CBA/J, and DBA/1J); intermediate (SWR/J, BALB/cJ, C57BL/10J, and DBA/2J); and sensitive (A/J, SJL/J/ C3H/HeJ, and C57BL/6J). The A/J strain was found to be most susceptible to develop rapid and severe DF, whereas the 129P3/J strain was the most resistant, showing little to no effects. These data support that genetics (host factors) do play a role in DF resistance and susceptibility and allow us initiate QTL mapping to identify specific genes contributing to DF resistance and susceptibility.

**Common founder mutations among Hispanic Patients with ataxia-telangiectasia (A-T).** *M. Mitui<sup>1</sup>, S. Castellvi-Bel<sup>1</sup>, G. Coutinho<sup>1,2</sup>, Y. Huo<sup>1</sup>, X. Sun<sup>1</sup>, R.A. Gatti<sup>1</sup>.* 1) Pathology, UCLA School of Medicine, Los Angeles, CA 90095-1732; 2) Instituto de Biofisica Carlos Chagas Filho, UFRJ, Brazil.

We have genotyped A-T families from Hispanic countries to determine the contribution of different ethnic groups to the present-day Hispanic population affected by A-T. We have studied a total of 66 new patients (22 Spanish, 21 Brazilians, 22 Hispanic-Americans, and 1 Chilean). Genomic DNA was isolated from whole blood and genotyped with four microsatellite markers within and flanking the ATM gene, an interval spanning ~1.4 cM. Alleles for each marker were standardized to sample CEPH 1347-02. We identified 31 haplotypes in Spain (4 recurring), 25 in Brazil (4 recurring), and 35 in Hispanic-Americans (3 recurring). Mutation screening was performed by PTT, SSCP, and dHPLC; this has allowed the identification of 70% of the alleles to date. A total of 55 different mutations were detected, the majority were truncations or splicing defects. Only two missense mutations were identified, both of them located within the kinase domain, affecting highly conserved amino acids. In most cases, shared mutations were carried on shared haplotypes, indicating common founder mutations. However, two Spanish mutations were observed on more than one haplotype: IVS21+1G>A on haplotypes [H] and [J], and 8977C>T on haplotypes [B] and [N]. These data might represent recurrent mutational events in the ATM gene. In Brazil, four haplotypes accounted for 48% (10 of 21) of the patients. Recurring haplotypes were observed in 59% of the of the Spanish A-T patients, 62% of the Brazilians, and 41% of the Hispanic-Americans. These data indicate that preliminary haplotyping greatly simplifies mutation identification in populations with recurring haplotypes.

**The frequency and molecular genetics of CF in Belarus.** *G.L. Tsukerman*<sup>1,2</sup>, *N.I. Mosse*<sup>2</sup>, *K.A. Mosse*<sup>2</sup>. 1) Reproductive Genetics Institute, Chicago, IL; 2) Institute for Hereditary Diseases, Minsk, Belarus.

A few years ago the frequency of delta-F508 mutation was studied in 2, 598 newborns alleles. The frequency of delta-F508 mutation heterozygote carriers in Belarus newborns population was revealed 0.014 or 1:72. As this mutation accounted for 63% of CF alleles in our patients, so CF incidence was to be 1:8108. Then a pilot whole population neonatal screening for CF was started. A successful two-tier screening strategy for CF using an initial estimation of IRT at cut-off 70 ng/ml followed by direct CF gene analysis was conducted. Sweat test was done in all the cases where IRT value was above of the cut-off. Totally 146 701 newborns were screened and 18 (eighteen) cases of CF were diagnosed. This means that the incidence of CF in newborns population of Belarus is 1:8150. The expected and observed incidence of CF in Belarus revealed absolutely the same numbers, which are far from 1:2500. 232 CF alleles were examined in Belarus patients. The samples were screened for the presence of the following mutations that were originally identified in European population: delta F-508, R334W, R347P, G542X, S549N, R553X, W1282X, N1303K. Analysis has shown that delta F-508 mutation covered 63% of CF chromosomes, R334W 0.4%, R347P 0.4%, G542X 1.3%, S549N 0.4%, R553X 0.4%, W1282X 0.9%, N1303K 2.5%. Recently, a large genomic deletion of CFTR gene that is frequently observed in Central and Eastern Europe was identified. The mutation, termed CFTRdel2, 3(21kb), deletes 21,080 bp spanning introns 1-3 of CFTR gene (T. Dork et. al., 2000) Our data show that the mutation is particularly common in Belarus - 4.3% of all CF chromosomes.



**Determination and Analysis of >30,000 SNP Allele Frequencies in Three Defined Populations and CEPH Pedigrees from SNPs Identified by The SNP Consortium.** *M.S. Phillips, V.M. Ankeney, D.W. Morris, J.F. Studebaker, M.A. Donaldson, J.A. Lathrop, F.-S. Kuo, A.V. Alfisi, G.A. Gelfand, M.G. Pohl, M.T. Boyce-Jancino.* Orchid BioSciences, Inc., Princeton, NJ.

In collaboration with The SNP Consortium (TSC), Orchid BioSciences has determined the allelic frequencies for more than 30,000 SNPs obtained from the TSC database. The SNP allelic frequencies are being determined on samples derived from three different defined populations and selected CEPH pedigrees. The three different populations consist of approximately 40 individuals from each of the Caucasian, African American and Asian (composed of 75% Japanese and 25% Chinese) populations. The ultimate goal of the project is to have information and functional assays for the development of useful SNP panels for at least 60,000 TSC SNPs screened against all 3 populations. Selection of the SNPs is genome wide with a slight emphasis on genes involved with drug metabolism and those, which are potential targets for drug development. Using Orchids proprietary high throughput single-base primer extension technology, SNP-IT™, individual genotypes have been determined for each of the samples in the respective populations. This equates to well over 7.2 million genotypes to be completed by the end of the project. Orchid is performing the SNP-IT™ genotyping on multiple ultra-high throughput platforms. One platform is Orchids completely automated SNPstream 25K system, which uses 384 well plates with a colorimetric readout. The other platform being utilized is Orchids SNPcode, which uses Affymetrix GenFlex™ chips coupled with multiplex PCR and primer extension to perform the analysis of 1000 SNPs at a time on a single sample. A selection of the 60,000 TSC SNPs is also being analyzed on 10 CEPH pedigrees (80 individuals) for the determination of SNP linkage and haplotypes within families. Analysis of the SNPs on the CEPH pedigrees will provide the initial components for a linkage disequilibrium (LD) map of the genome. The results of these studies will form the basis for the first defined human SNP map and generate the resources necessary to conduct genome-wide association studies.

**The effect of sequencing errors on the frequency spectrum of segregating mutations.** *J.D. Wall.* Dept Organismic & Evol Biol, Harvard Univ, Cambridge, MA.

The distribution of frequencies of single nucleotide polymorphisms (called the "frequency spectrum") has often been used to make inferences about human demographic history. For example, an excess of rare polymorphisms is commonly interpreted as evidence for a recent increase in the human population size. However, confounding factors, such as natural selection, ascertainment bias or experimental error, can make it difficult to interpret the observed patterns of polymorphism. Here we present some simple models of sequencing error, and explore through simulations what effect they have on the expected frequency spectrum. We find that ignoring the effects of sequencing errors when they actually exist can lead to erroneous inferences regarding human demographic history. If genotyping preascertained polymorphisms has a significantly lower error rate than sequencing, it may be more informative to first ascertain polymorphisms in a sample of size 2 or 3, then determine the frequencies from a much larger sample.

**Frequency of CYP3A and MDR-1 polymorphisms in Healthy Volunteers and Commercial Ethnicity Panel.** S.A. Henry<sup>1</sup>, E.A. Foot<sup>2</sup>, G.P. Sreekumar<sup>1</sup>, V.D. Schmith<sup>2</sup>, S.A. Asquith<sup>1</sup>, T. Saunders<sup>1</sup>, D. Dow<sup>1</sup>, R. Hussein<sup>1</sup>, B. Bahari<sup>1</sup>, Z. Xue<sup>1</sup>, D.S. Montgomery<sup>1</sup>, M. Dickins<sup>3</sup>, I.J. Purvis<sup>1</sup>, M.J. Stubbins<sup>1</sup>, A.J. Yeo<sup>1</sup>. 1) Genetics Research, GlaxoSmithKline, RTP, NC; 2) Clinical Pharmacology & Experimental Medicine, GlaxoSmithKline, Brentford, Middlesex, TW8 (ET, UK and RTP, NC 27709 US; 3) DMPK, GlaxoSmithKline, Brentford, Middlesex, TW8 9ET, UK and RTP, NC 27709 US.

Understanding the presence and frequency of genetic variation in the enzymes and transporters involved in the absorption, distribution, metabolism and excretion of drugs is of critical importance in interpreting variability in drug response. Knowing the frequency of these variants in healthy volunteers and ethnic groups will allow extrapolation of data obtained from a predominantly Caucasian population in Phase I studies to other populations. CYP3A4/5 and MDR1 are of particular interest due to their roles in metabolizing/transporting the majority marketed drugs. In this study, following appropriate informed consent, DNA samples were collected from 129 healthy Caucasian subjects in phase I clinical trials. A panel of taqman assays for 5 variants in CP3A4,3A5 and MDR1 were developed. These assays were used to genotype the clinical trial samples and to determine allele frequencies in 3 ethnic populations using 50 commercially available DNA samples from each of Caucasian(CC),African-American(AA)and Hispanic (H)populations.All markers genotyped were in Hardy-Weinberg equilibrium. Frequencies for the minor alleles in CYP3A4 A816G and CYP3A5 T-369G were significantly lower in CC(1% and 6%)compared to AA(71% and 60%)and H(11% and 24 %).The frequency of the T allele in MDR1 C3435T was significantly lower in AA (17%)compared to CC(51%)and H(40%),while the frequency of the G allele in MDR1 T2793G was significantly higher in AA(94%)compared to C(55%)and H(63%). This data needs to be considered when conducting studies in different parts of the world. The allele and genotype frequencies in the CC clinical trial subjects were not significantly different from the CC from the ethnicity panel indicating that these healthy subjects are representative of the general population.

**Pharmacogenomics study of glucocorticoid sensitive and resistant asthma predicts glucocorticoid responsiveness with high accuracy.** *H. Hakonarson<sup>1</sup>, U.S. Bjornsdottir<sup>2</sup>, E. Halapi<sup>1</sup>, F. Zink<sup>1</sup>, H. Helgadóttir<sup>1</sup>, I. Bjarkarson<sup>1</sup>, T. Arnason<sup>1</sup>, F. Thorarinsson<sup>1</sup>, A.S. Gudmundsdóttir<sup>1</sup>, S. Ingvarsson<sup>1</sup>, L. Amundadóttir<sup>1</sup>, M. Andresdóttir<sup>1</sup>, E.A. Adalsteinsdóttir<sup>1</sup>, D. Gislason<sup>2</sup>, T. Gislason<sup>2</sup>, M. Gurney<sup>1</sup>, J. Gulcher<sup>1</sup>, K. Stefansson<sup>1</sup>.* 1) deCode Genetics, Inc, Reykjavik, Iceland; 2) Department of Pulmonary Medicine, National University Hospital, Reykjavik, Iceland.

For most drugs, the genetic variations that determine clinical drug response remain to be uncovered. We compared gene expression profiles in freshly isolated peripheral blood mononuclear (PBM) cells from glucocorticoid (GC)-sensitive (S) and GC-resistant (R) asthmatics using gene array technology. We searched for genes with sufficient power to predict GC-S (responders) and GC-R (non-responders) asthmatics with high accuracy, using a naive Bayesian classifier. Total RNA was extracted from unstimulated PBM cells and from cells treated with IL-1b/TNF $\alpha$  in the absence and presence of GC treatment. Expression of multiple genes was examined using gene array technology in 60 asthmatic patients and 15 control subjects. Collectively, 2.4 million datapoints were analysed. The classifier was trained on a cohort of 14 GC-R and 14 GC-S patients. Fifty genes were selected by the classifier that predicted the correct GC-response phenotype with over 80 % accuracy ( $p < 0.05$ ). When the algorithm was applied to a second cohort of 30 asthmatics, the predictive accuracy of the classifier to predict GC-R asthma was 86%. The expression profile in control subjects was similar to that of GC-S patients. Preliminary results in 24 asthmatics indicate similar predictive accuracy in terms of leukotriene antagonist response. We conclude that this study is the first to characterize gene expression profiles in freshly isolated PBM cells that differ in GC-R and GC-S asthmatics and provided sufficient power to predict clinical response to GC with high accuracy. The genes identified are currently being searched for SNPs. This type of pharmacogenomics platform may lead to the development of novel therapeutic strategies and diagnostic tests.

**TWO NEW RECURSIVE LIKELIHOOD CALCULATION METHODS FOR GENETIC ANALYSIS.** *A. Yuan, G.E. Bonney.* National Human Genome Center, Howard University, Washington , DC.

Recursive likelihood calculations for genetic analysis of pedigree data employ variations of the Elston-Stewart (ES) and the Lander-Green (LG) algorithms. With the ES algorithm, the number of loci may be limited but not the pedigree size. With the LG algorithm, the reverse is the case, i.e., the pedigree size may be limited but not so much the number of loci. We introduce two new algorithms for likelihood models over pedigree. In one algorithm, the likelihood function is factored into a product of all father-mother-offspring-trios. This allows for great gains in computational speed. In the second algorithm, the likelihood function is, in effect, approximated by a product over all persons in the pedigree, with each factor having only one summation over unobserved genotypes. This is faster than the former method, and yields very good results if the effects of the underlying loci are not large. The methods are implemented in G.E.M.S., and studies in univariate trait are presented.

**Group sequential methods in genotype-disease association studies that are unlikely to reject the null hypothesis.**

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**Introduction:** Molecular epidemiological association studies use valuable biosamples and incur material and labor costs. Statistical methods for early genotyping termination of studies that cannot reject the null hypothesis may conserve biosamples and costs. Group sequential methods (GSM) allow clinical trials to be terminated early for significant differences between treatments. We hypothesized that a group sequential boundary (GSB), that compares a test statistic to values for acceptance and rejection of the null hypothesis, allows early genotyping termination if the null hypothesis were true. **Methods:** We simulated the GSB in a case-control study of GST m and q genotypes and prostate cancer (CEBP, 8:283-7, 1999). This study reported a positive association of GST q null (OR= 1.61, p=0.0068, N=720) and a negative association of GST m null (OR=.998, p=0.991, N=630). Acceptance and rejection boundaries were defined using EAST™ software with an overall  $\alpha$  and  $\beta$  of 0.05 and 0.80. These boundaries were used in a simulation of 1000 repetitions of case/control genotyping. Cases and controls were randomly sampled without replacement, in proportion to their relative frequencies, and in groups of 90 to simulate genotype acquisition from PCR analysis. A  $\chi^2$  test statistics was calculated for each group of 90 samples (a "look") and compared to predefined boundaries for study termination. **Results:** On average, GST m genotyping terminated at 3.8 looks. GST m genotyping was not stopped at looks 1 and 2, but was terminated in 354 simulations at look 3, in 476 simulations at look 4, in 152 simulations at look 5, and 17 simulations at look 6. The GSB decreased the average number of genotyped biosamples to 426, or 67.7% of the total sample. GST q genotyping was stopped in 12.2% of simulations. **Conclusion:** The GSB allows early termination of genotyping when the null hypothesis is true. Early termination of GST m genotyping saves an average 32.2% of biosamples from unnecessary genotyping. The GSB has an acceptable frequency of inappropriate termination. Funded by the Doris Duke Foundation.

**Association of Candidate Polymorphisms with Diabetic Retinopathy.** *J.P. Burke<sup>1</sup>, V.S. Pankratz<sup>1</sup>, D.J. O'Kane<sup>2</sup>, P.J. Dyck<sup>3</sup>*. 1) Health Sciences Research, Mayo Clinic, Rochester, MN; 2) Laboratory Medicine and Pathology; 3) Neurology.

The incidence of diabetes is increasing rapidly in the United States. Individuals with type 1 (dm1) or type 2 (dm2) diabetes are at a substantially increased risk of developing complications such as neuropathy, nephropathy and retinopathy. Although blood glucose is a major risk factor for diabetes complications, it fails to explain a majority of the severity of complications. A number of potential etiological pathways have been identified for the development of diabetic complications. These include the renin-angiotensin system, vascular homeostasis, advanced glycation end products, and the polyol pathway. Our initial goal is to examine the association of 7 candidate polymorphisms identified within these pathways with the development and severity of diabetic complications in the Rochester Diabetic Neuropathy Study (RDNS), a population based prospective study of approximately 400 individuals with diabetes living in Rochester, MN on January 1, 1986. Our long-term goal is to construct improved models to predict those at increased risk of developing diabetic complications. Preliminary analyses, including 96 dm1 and 162 dm2, have been conducted. Standardized criteria were used to define dm1, dm2, neuropathy, nephropathy and retinopathy. The frequency of neuropathy, nephropathy and retinopathy were 58.3%, 22.1% and 83.0% in dm1 individuals, respectively, and 48.2%, 44.4% and 65.6% in dm2 individuals, respectively. A significant association was found between apoE4 T3937C SNP and the presence of retinopathy in individuals with dm1 (T/T: 70.2% vs. 41.7%,  $P=0.047$ ). In addition, a significant association was found between aldose reductase C106T SNP and the presence of retinopathy in individuals with dm2 (C/C: 30.4% vs. 55.6%,  $p=0.018$ ). Lastly, a borderline association was found between aldose reductase C106T SNP and the presence of nephropathy in individuals with dm1 (T/T: 22.2% vs. 5.0%,  $p=0.080$ ). We conclude that there were significant associations between polymorphisms in the apoE4 and aldose reductase genes and presence of diabetic complications, particularly retinopathy. These associations may lead to improved prediction models of diabetic complications.

**Genetic variation in apolipoprotein D affects the risk of Alzheimer's disease in African Americans.** *P.P. Desai<sup>1</sup>, H.C. Hendrie<sup>2</sup>, R.M. Evans<sup>3</sup>, J.R. Murrell<sup>4</sup>, S.T. DeKosky<sup>1,5</sup>, M.I. Kamboh<sup>1,5</sup>*. 1) Human Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Psychiatry, Indiana Univ, Indianapolis, IN; 3) Neurology, Indiana Univ, Indianapolis, IN; 4) Pathology & Laboratory Medicine, Indiana Univ, Indianapolis, IN; 5) Psychiatry, Univ of Pittsburgh, Pittsburgh, PA.

Several genetic and environmental factors have been implicated in the onset and pathology of Alzheimer's disease (AD). Among genetic factors, apolipoprotein E (APOE) alters susceptibility to AD. Although African Americans appear to have a higher prevalence of AD than Whites, a weaker association has been seen between *APOE\*4* and AD among African Americans. These observations suggest that additional unidentified genetic and environmental factors may explain the increased risk of AD among African Americans. Recently, the product of a new candidate gene, apolipoprotein D (APOD), has been shown to be involved in the processes of neuroregeneration and neurodegeneration. We have identified several polymorphisms in the APOD gene, which are unique to populations of African ancestry. In the present study we examined the role of four APOD genetic variants (Intron 1, codons 36, 108, 158) in modifying the risk of AD in 70 African Americans with AD (mean age 81.43±0.81) and 163 nondemented subjects (mean age 77.78±0.55) from a population-based cohort in Indianapolis. Although the *APOE\*4* allele frequency was higher in cases than controls (0.293 vs. 0.199; p=0.035), the age and sex adjusted odds ratio (OR) was not significant (OR=1.52; 95%CI: 0.84-2.74; p=0.17). Two of the APOD polymorphisms (Intron 1 and codon 36) showed an association with AD. The *Intron 1\*2* allele was associated with a higher AD risk with an age, sex and APOE adjusted OR of 2.29 (95%CI: 1.19-4.43; p=0.013). The frequency of codon 36/GT genotype was higher in AD cases than controls (4.3% vs. 1.2%), with an adjusted OR of 4.24 (95%CI: 0.66-27.14; p=0.13). Our data suggest that the risk of AD among African Americans is modified by genetic variation in APOD. Larger population-based or case-control studies, together with functional studies may help to elucidate the structure-function relationship of APOD genetic variants in the etiology of AD.



**Lack of association between thermolabil variant C677T in MTHFR gene and neural tube defects in the State of Yucatan, Mexico.** *L.J. Gonzalez-Herrera<sup>1</sup>, M.G. Garcia-Escalante<sup>1</sup>, I. Castillo-Zapata<sup>1</sup>, J. Canto-Herrera<sup>1</sup>, J.M. Ceballos-Quintal<sup>1</sup>, D. Pinto-Escalante<sup>1</sup>, A. Gonzalez-Del Angel<sup>2</sup>.* 1) Laboratorio de Genetica. CIR, Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico; 2) Departamento de Investigacion en Genetica Humana. Instituto Nacional de Pediatria. Mexico,D.F.

Neural tube defects (NTD) are the most prevalent isolated congenital malformations (6.6 in 1000) in the State of Yucatan, Mexico. The thermolabil variant C677T in MTHFR gene leads to altered homocysteine metabolism and it is considered an associated risk factor for NTD. However, the association has been controversial and it seems to be dependent of the C677T variant frequency, which is heterogeneous among populations. So, The objective of this work was to determine the association of thermolabil variant C677T in MTHFR gene with NTD in the State of Yucatan, Mexico, where NTD are highly prevalent and with Mayan native background. A case-control study was performed with 60 newborn subjects affected with NTD (52 spina bifida, 3 anencephaly and 5 encephaloce) and 100 control healthy volunteers from yucatecan general population as well as 54 mothers of NTD children and 56 control mothers who had healthy offspring. The C677T variant in MTHFR gene was identified by PCR technique and restriction with HinF I enzyme. Among NTD subjects, odds ratios for the variant C677T homozygosity and heterozygosity were 1.35 (0.48-3.8) and 1.45 (0.56-3.82) respectively, relative to wild type homozygotes and 1.13 (0.70-1.83) for C677T allele relative to wild type allele, whereas for mothers, odds ratios were 1.21 (0.35-4.15), 2.01 (0.67-6.11) and 1.4 (0.59-1.84), respectively. Genotype frequencies among control groups were according to Hardy-Weinberg expectations ( $p > 0.33$ ). No statistically significant associations were observed for any genotype or allelic condition ( $p > 0.05$ ), suggesting that the thermolabil C677T variant is not an associated risk factor for having NTD neither to have affected offspring in the yucatecan population. Gene-gene or gene-environment interactions should be evaluated since they might increase the risk for NTD in the studied population.

**SNP genotyping on genome wide amplified DOP-PCR template.** *S.F.A. Grant, R. Tolle.* Genetic Epidemiology, LION Bioscience, Heidelberg, Germany.

DOP-PCR (degenerate oligonucleotide primed polymerase chain reaction), employs a degenerate primer (5'-CCGACTCGAGNNNNNNATGTGG-3') to produce unspecific uniform amplification of DNA. This approach has been successfully applied to microsatellite genotyping<sup>1</sup>

With the increasing demand for higher throughput single nucleotide polymorphism (SNP) genotyping, the quantity of genomic DNA often falls short of the number of assays required. We investigated the use of DOP-PCR to generate template for our SNP genotyping methodology of template-directed dye-terminator incorporation assay with fluorescence polarization (FP-TDI) detection. We compared the original cycling protocol<sup>1</sup> with the methodology outlined by Roche in their 'DOP PCR Master' kit for in situ hybridization and comparative genome hybridization (CGH). Results were analysed with respect to feasibility, allele loss, genotyping accuracy and storage conditions.

The original cycling procedure gave an error rate of approximately 4%: 96°C 8min initial denaturation, 8 cycles of 93°C 1min, 30°C 1min, and 72°C 3min, and then 28 cycles of 93°C 1min, 60°C 1min, and 72°C 3min. In comparison the Roche cycling protocol, which prolongs extension times, yielded an error rate lower than 1%: 95°C 5min initial denaturation, 5 cycles of 94°C 1min, 30°C 1.30min, ramping to 72°C over 3min (3.5°C/15s) and 72°C 3min and then 35 cycles of 94°C 1min, 62°C 1min and 72°C 2min (and increasing by 14s each subsequent cycle) and a final extension step of 72°C 7min.

Degradation of the DOP-PCR stored at -70°C has been observed. By adding vector DNA in excess (100ng to a 10ul PCR product) to act as a template for residual nuclease activity, degradation is minimised.

In conclusion, we have successfully used DOP-PCR to amplify our genomic DNA collection for subsequent SNP genotyping. The cycling protocol proposed by Roche produced fewer typing errors than the original methodology.

<sup>1</sup>V.G. Cheung and S.F. Nelson. PNAS 93; 14676-14679 (1996).

**Identification of three novel polymorphisms in the MJD1 gene and their frequency study in a Portuguese population.** *M.C. Costa<sup>1</sup>, C. Santos<sup>1</sup>, A. Ferro<sup>1</sup>, M. Santos<sup>1</sup>, J. Sequeiros<sup>1,2</sup>, P. Maciel<sup>1,3</sup>.* 1) UnIGENE, IBMC, Univ. Porto, Portugal; 2) Depto. de Estudos das Populações, ICBAS, Univ. Porto; Portugal; 3) Inst. Sup. Ciências da Saúde-Norte, Portugal.

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder of late onset, caused by an expansion of a (CAG)<sub>n</sub> tract in the MJD1 gene. We made a multiple alignment sequence (BLAST 2 sequences) between known cDNA variants transcribed by the MJD1 gene and a BAC clone of human genomic DNA from chromosome 14, BAC R-529H20. This comparison suggested the possible existence of six unknown single nucleotide intragenic polymorphisms (SNPs) at variable positions in the MJD1 gene. In order to confirm this, we have studied 76 individuals from a Portuguese control population, using PCR amplification and SSCP analysis for each potential SNP. For four of the possible polymorphisms we had no variants in the population studied, but we have confirmed the existence of three novel polymorphisms:  $\text{GTT}^{527}/\text{GTC}^{527}$ ,  $\text{C}^{1178}/\text{A}^{1178}$  and  $\text{A}^{1294}/\text{G}^{1294}$ . The polymorphism  $\text{GTT}^{527}/\text{GTC}^{527}$  (Val/ Val) is located in the coding region, and the others  $\text{C}^{1178}/\text{A}^{1178}$  and  $\text{A}^{1294}/\text{G}^{1294}$  are located in the 3'non-coding region of cDNA variants of MJD1 gene, MJD2-1 and MJD1-1, respectively. The frequencies on the population studied for genotypes for  $\text{GTT}^{527}/\text{GTC}^{527}$  were: T/T- 0,18, C/C- 0,42, and T/C- 0,40; for  $\text{C}^{1178}/\text{A}^{1178}$  were: C/C- 0,04, A/A- 0,71 and C/A- 0,25; and for  $\text{A}^{1294}/\text{G}^{1294}$  were: A/A- 0,03, G/G- 0,71 and A/G- 0,26. All these novel SNPs are in Hardy-Weinberg equilibrium. These intragenic polymorphisms can be useful (1) for the study of the origin of the MJD mutation(s); (2) for the study of recombination events (proposed by several authors to be involved in the instability of trinucleotide repeats); (3) for distinction of chromosomes with alleles of identical size in genetic tests (homoallelism); (4) and for the study of genetic modifiers of the in the region flanking the MJD1 gene.

**Genetics studies in isolated Amerindian community: Warao in the Orinoco delta.** A. Arends, M. Chacin T., M. Bravo Urquiola, M. Alvarez, T. Arends de O., A. Ciarello, A. Cardenas. Hematology, Hosp Universitario de Caracas, Caracas, Venezuela.

The Waraos are an Amerindian tribe of 20,000 subjects, who live in the Orinoco River Delta, in the northeastern part of Venezuela, very close to the Atlantic Ocean. They are dedicated to fishing and swamp scavenging and show very strong cultural differences with the surrounding Amerindian groups. Two hundred individuals belonging to two small Warao communities: Cangrejito and Winiquina were studied to determine if there had been any admixture with other populations. Hemoglobin analysis were carried out by HPLC and electrophoresis, Hb A2 was measured by cation exchange column and Hb F by alkali denaturation. Hematological parameters were determined by automatic cell counter (Coulter).  $\beta$  thalassemia mutations were determined by ARMS-PCR and  $\beta$  globin gene haplotypes frequency by PCR-RFLP of 5 polymorphic sites in 170 chromosomes. We found a higher degree of anemia in children, and most of them with nutritional deficit, 2 carriers of gen  $\beta^s$ , 4 with high levels of Hb A2 (3.5-5%), two of them carried the CD39 mutation. HPFH, found 25 years ago, was present in 7 members of a large kindred, with 2-8% of Hb F. Haplotype 2 was the most frequent (26%), followed by haplotype 6 (22%), and the non-reported to our knowledge, atypical Warao haplotype in a frequency of 13%, 2 individuals were homozygous for this haplotype. The finding of 8 alleles with haplotype 3 in apparently non-related individuals and the presence of two alleles with the CD39  $\beta$  thalassemia mutation are clear evidence of recent admixture. In earlier studies, 30 years ago, of some genetic markers in this Amerindian tribe, there had not been any indication of admixture with caucasoid and african subjects. The Warao occasionally venture out of the delta to trade overseas with Trinidad and Tobago, and upstream towards more populated and mixed Venezuelan villages. We postulate that the high frequency of the Warao haplotype in this isolated community is possible due to genetic drift, because of the high degree of endogamy among these small communities. Financial Support: CONICIT S1-95000696, CDCH-UC.

**Human progesterone receptor gene polymorphisms and endometrial cancer risk.** *G.M. Boezen<sup>2</sup>, S.E. Hankinson<sup>1,3</sup>, P.J. Lescault<sup>1</sup>, G.A. Colditz<sup>1,3</sup>, D.J. Hunter<sup>1,3</sup>, I. De Vivo<sup>1,3</sup>.* 1) Dept Medicine, Channing Lab, Harvard Medical Sch, Boston, MA; 2) Dept of Epidemiology, Univ of Groningen, The Netherlands; 3) Dept of Epidemiology, Harvard Sch of Public Health, Boston, MA.

The progesterone receptor gene is a member of the steroid receptor super-family of transcription factors. The gene is mainly expressed in the female genital tract, the breast and the brain. Endometrial cancer is a model system in which to study PR variants, given that the role of progesterone is to act primarily as an antagonist to estrogen-mediated cell proliferation. Therefore, variants within the PR receptor may be of particular importance in the endometrium. Blood was collected in 1989 from participants of the Nurses' Health Study cohort. Using the human genome working draft sequence (GI:8570374), we screened the 8 exons, splice sites and promoter region. All 187 documented cases of invasive endometrial cancer through 1996, and 561 matched controls, were genotyped using various methods. We inferred haplotypes based on wild type (WT) vs. variant (V) (het&homozygotes) combinations of the observed polymorphisms, and in addition by using the EM-algorithm. Logistic regression was performed, for all inferred haplotypes separately, controlling for potential confounders. We found a total of 6 variable sites. Novel variants were found in the promoter region (+44C/T, +332G/A), and in the coding region S344T, G393G, V660L, H770H. We also confirmed the Alu306 polymorphism. S344T, V660L, H770H and Alu306 were in complete linkage disequilibrium ('framework', FrW). Prevalence of variant carriers was (cases vs. controls) 12% vs. 14% for +44C/T; 15% vs. 15% for +332G/A, 61% vs. 56% for G393G, and 27% vs. 30% for FrW. Subjects with V332G/A / WTFrW (OR=1.66; 95%CI=0.94-2.92) or V332G/A / VFrW (1.24; 0.38-4.00) were somewhat more likely to be cases compared to WT332G/A / WTFrW; those with VG393G / WTFrW (1.21; 0.78-1.88) or VG393G / VFrW (1.03; 0.53-2.01) were slightly more likely to be cases compared to WTG393G / WTFrW. Polymorphisms S344T, V660L, H770H and Alu306 are in complete linkage disequilibrium, thus constructing a haplotype-framework. This framework is not associated with endometrial cancer risk.

**The 165/166delGG@insC mutation in the paracellin-1 gene is a common polymorphism.** D.E.C. Cole<sup>1,2</sup>, Z. Liu<sup>2</sup>, B.Y.L. Wong<sup>2</sup>, C. Wei<sup>2</sup>. 1) Dept of Laboratory Medicine & Pathobiology, University of Toronto, Toronto ON, Canada; 2) TGH Genetic Repository, University Health Network, Toronto ON, Canada.

Paracellin-1 (PCLN1), a member of the claudin tight junction protein family, has been identified as a crucial component mediating paracellular divalent cation conductance in the thick ascending limb of the loop of Henle. *PCLN1* mutations have been identified in patients with the recently delineated hypomagnesemia-hypercalciuria syndrome. In a search for variants of the *PCLN1* gene, we sequenced all 5 exons using published intronic primers. In 16 young healthy Caucasian adults, no variation was detected in exons 2 to 5. In exon 1, we found a 165/166delGG@insC mutation in 7 of 16 samples (6 heterozygotes; 1 homozygote). For all 8 variant alleles, there was an associated IVS1+10T@C SNP. To determine allele frequencies in different ethnic groups, we genotyped leukocyte DNA from 70 Caucasians, 50 Afro-Americans, and 45 Asians using PCR and *Bsa*JI digestion. There was no evidence of Hardy-Weinberg disequilibrium. The variant allele frequencies in Caucasian (0.23), Afro-American (0.10), and Asian (0.01) groups were significantly ( $p < 0.01$ ) different from one another. Given the published start site, this polymorphism would induce a frameshift at codon 55 (Arg55fs) and a stop codon at amino acid position 71, resulting in an aberrant protein product truncated just prior to the first transmembrane domain. Although the 165/166delGG@insC mutation has been suggested to be pathogenic, the high frequency of this allele in three ethnic groups and the existence of homozygotes in the general population make it unlikely that it is deleterious. We speculate that the second in-frame methionine codon (position 71) with a suitable Kozak consensus sequence is the preferred start codon *in vivo*. This position is homologous to the start site of rat, mouse, and bovine paracellins, and other claudins. Further study is required to determine the nature of the transcripts and the functional translation start site(s) *in vivo*.

**Polymorphisms of the fibrinogen gene : their association with cord blood fibrinogen levels and family history of coronary artery disease in Singapore neonates.** *S.C. Quek, C.K. Heng, S. Hong, P.S. Low, N. Saha.* Dept Pediatrics, National Univ Singapore, Singapore.

**Introduction:** Elevated plasma fibrinogen is known to be a risk factor of coronary artery disease (CAD) in adults. We report the results of cord fibrinogen studies in term infants of the 3 races (Chinese, Malays, Asian Indians) in multiracial Singapore. **Patients and Methods:** Cord fibrinogen levels of healthy term newborns of the 3 races were assayed by the Clauss's method. Subjects with a family history (FH) of CAD in 1 or more first/second degree relative(s) were categorized FH+. The association of FH, 5 polymorphisms of the fibrinogen gene (-148C/T, +1689T/G, *BclII*, *TaqI*, T312A) with fibrinogen levels of the 3 ethnic groups was examined. **Results:** Cord fibrinogen levels (mg/dl) were significantly lower in the Indians ( $117\pm 60$ ) compared to the Chinese ( $128\pm 54$ ) and Malays ( $128\pm 63$ ) at  $p=0.006$  after adjusting for the confounding effect of birth weight and gestational period. There were also significant differences in cord fibrinogen levels with respect to FH of CAD in Chinese and Malay babies, but not in Indians. The mean cord fibrinogen levels in those with negative and positive FH were 134.40/113.12 ( $p<0.0005$ ) and 134.97/115.36 ( $p=0.007$ ) in Chinese and Malays respectively. Given that fibrinogen is a risk factor for CAD, the unexpectedly higher levels in the FH- neonates and the high-risk Asian Indians was contradictory to findings in adults and puzzling. All polymorphisms studied were not significantly associated with fibrinogen levels. However, the rare allele frequencies (FH- vs FH+) for the +1689T/G (0.12 vs 0.31) and T312A (0.33 vs 0.24) sites were significantly higher for the Indians ( $p<0.0001$ ) and lower for the Malays ( $p<0.05$ ) in the FH+ group, respectively. **Conclusions:** Significantly lower cord fibrinogen levels were found in Chinese and Malay babies with a FH of CAD compared to the Indians. Although the fibrinogen gene polymorphisms were not significantly associated with fibrinogen levels, their allelic frequencies of the +1689T/G and T312A sites were significantly different between FH+ and FH- neonates in the Indian and Malay babies, respectively.

**Testing of the origin of the bS gene in a non-negroid venezuelan population.** *L. Pineda*<sup>1</sup>, *H. Chirino*<sup>2</sup>, *L. Borjas*<sup>1</sup>, *W. Delgado*<sup>1</sup>. 1) Molecular Genetic Laboratory, Medical Genetic Unit, University Zulia, Zulia, Venezuela; 2) Medicine Faculty, Francisco of Miranda University, Falcon, Venezuela.

The studies carried out on the distribution of the bS gene in the world has qualified as african negroid marker. In Venezuela, the presence of bS gene has been correlated with an important contribution of african people to the genetic composition of those populations, however, at the northwest of the country there is a non negroid population named Toas Island, where 1 of 8 people is carried of the bS gene. Since this gene is related in disequilibrium of linkage with 5 haplotypes of the b-globin cluster, 4 of them, have been predominantly found in african continent, the aim of this research was to clarify about the origin of the bS gene in the Toas Island using these haplotypes. Five polymorphic restriction sites of the b-globin cluster were characterized by PCR: HincII-5'e, HindIII-Gg, HindIII-Ag, HincII-fb and HincII-3'fb in 87 members (parents and offspring) of 22 families that were segregating the bS gene (AAxAA: 17 and AAxAS:5). Segregation analysis permitted to identify the bS and bA chromosomes. The 61.36%(54/88)of the haplotypes were atypical and, only 6.8% were african haplotypes:(Benin:3, Senegal:2 and Bantu:1). The atypical haplotype ---+- was found linked to the bS gene in the 51.86% and like to african haplotypes (22.2%)was not present in bA chromosomes. This results and the historic documentation about this population are not in concordance with the findings in others regions of the american continent in relation to the african origin of the bS gene, on the contrary, they support the autochthonous origin of the bS mutation in Toas Island.



**Novel O6-methylguanine-DNA methyltransferase single nucleotide polymorphisms (SNPs) detected among healthy individuals from the Swedish normal population.** *A. Platz, S. Ma, K. Smoczynski, J. Hansson, U. Ringborg, S. Egyhazi.* Department of Oncology and Pathology, Cancer Centre Karolinska, Karolinska Hospital Stockholm, Sweden.

The DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) is involved in the cellular defense against alkylating agents. Genetic alterations in the MGMT gene may impair the cellular capacity to remove alkyl groups from the O6-position of guanine, raising mutation rates and as a consequence increase the risk of cancer, whereas genetic alterations leading to increased MGMT activity may contribute to intrinsic resistance to alkylating drugs. We assessed genetic variation in the 5'-noncoding region and the 5 exons of the MGMT gene among 76 healthy volunteers from the Swedish population with the aid of single strand conformation polymorphism (SSCP) combined with nucleotide sequence analysis. In total eleven SNPs were detected, 5 in the 5'-noncoding region, one in exon 1, two in exon 3 and three in exon 5. Six of these SNPs are novel, five were located in the 5'-noncoding region and one in exon 5. The registered SNP frequencies ranged from 0.7 to 36%. Not much is known on the functional significance of MGMT SNPs. Comparative studies on MGMT variant frequencies among patient populations and in vitro studies of the cloned variants are in progress in order to clarify the clinical impact of the recognized genetic variability.

**Single-Nucleotide Polymorphisms at Several Cancer Susceptibility Genes Do Not Conform to the Infinite Site model of Mutation.** *A. Renwick<sup>1</sup>, P. Bonnen<sup>2</sup>, D. Triikka<sup>2</sup>, D. Nelson<sup>2</sup>, R. Chakraborty<sup>3</sup>, M. Kimmel<sup>1</sup>.* 1) Dept Statistics, Rice Univ, Houston, TX; 2) Dept Human Molec Genetics, Baylor Coll Med, Houston, TX; 3) Human Genetics Ctr., Univ Texas at Houston, Houston, TX.

The Infinite Site Model (ISM) is generally employed to explain the observed diversity at the human Single-Nucleotide Polymorphism (SNP) sites. However, it was also observed that there exists a considerable variability of diversity along the human genomic sequence. In addition, the SNP sites are discovered using procedures, which tend to leave out the less frequent variants, leading to a possible ascertainment bias in site-specific estimates of diversity. We examine the discovery process, assuming a model, which includes the ISM as a special case. We assume that full sequencing of a genome region is carried out in a limited discovery sample of chromosome. Once this is done, the primers developed are used to type a larger sample of chromosomes. By fitting our model, not only we can estimate the extent of ascertainment bias, but also conclude, which version of the model fits the data best. Application of this theory to SNP polymorphisms at over 50 sites encompassing five cancer-susceptibility genes (ATM, BLM, BRCA1, RECQL and WRN) suggests that: (i) SNP data within each of these genomic regions are best approximated by a model different from the ISM and (ii) the observed variability among different regions is unlikely to be due to differences in discovery procedures. We provide adjusted estimates of genetic diversity at SNP sites. The absence of fit to the ISM within specific genomic regions indicates possible influence of one or more factors, such as reverse or recurrent mutation, selection and recombination. (Research supported by NIH grants GM 58545 and CA75432).

**Four polymorphisms in the angiotensinogen gene and risk of hypertension and cardiovascular disease. The Copenhagen City Heart Study.** A.A. Sethi<sup>1, 2</sup>, B.G. Nordestgaard<sup>1, 2</sup>, H. Sillesen<sup>2</sup>, R. Steffensen<sup>2</sup>, G. Jensen<sup>2</sup>, A. Tybjaerg-Hansen<sup>1,2</sup>. 1) Department of Clinical Biochemistry; 2) University of Copenhagen, Denmark.

The angiotensinogen gene has been associated with hypertension, ischemic heart disease (IHD) and ischemic cerebrovascular disease (ICVD). We investigated the hypothesis that 2 promoter polymorphisms (A(-20)C and G(-6)A) along with 2 previously described polymorphisms (T174M and M235T) in the angiotensinogen gene, alone, combined, or as haplotypes, were associated with hypertension, IHD, or ICVD. In three case-control studies, 4950 individuals with hypertension were compared with 4234 individuals from the general population without hypertension, and 1805 patients with IHD and 920 patients with ICVD were compared with 7965 controls from the general population. Relative allele frequencies of -20C and -6A in the general population were 0.15 and 0.40. When combining the four polymorphisms, we identified 8 different haplotypes in 9191 individuals from the general population. The following results are based on the 6 most frequently observed haplotypes, and the ten most frequent genotype combinations (<sup>31</sup>). Systolic and diastolic blood pressure along with pulse pressure did not change as a function of either promoter polymorphism or the ten genotype combinations of all four polymorphisms in women and men. On multifactorial logistic regression analyses, women homozygous for the (-6)A mutation versus non-carriers had an odds ratio for hypertension of 1.26(95% confidence interval, 1.03-1.55), which increased to 1.52(1.11-2.07) if they in addition were homozygous for 235Thr, and to 1.72(1.02-2.90) if they also were heterozygous for (-20)C. No significant association was found between genotype and hypertension in men, nor between haplotypes and hypertension in either gender. On multifactorial logistic regression analyses, neither promoter polymorphism, alone, in combination with T174M and M235T, or as haplotypes predicted IHD or ICVD. In this study, double homozygosity for (-6)A and 235Thr along with heterozygosity for (-20)C is a risk factor for hypertension in women but not in men. The angiotensinogen gene mutations do not predict IHD or ICVD.

**Frequency of C677T MTHFR Mutation in the Yucatecan and Mayan native populations.** *G.M.G. Garcia-Escalante<sup>1</sup>, L.J. Gonzalez-Herrera<sup>1</sup>, J.M. Ceballos-Quintal<sup>1</sup>, I. Castillo-Zapata<sup>1</sup>, D. Pinto-Escalante<sup>1</sup>, A. Gonzalez-Del Angel<sup>2</sup>.* 1) Laboratorio de Genetica, UADY Merida, Yucatan, Mexico; 2) Departamento de Investigacion en Genetica Humana. INP, Mexico D.F.

The homozygosity for the MTHFR 677T mutation has been considered an associated risk factor for altered folate metabolism and transport diseases like cardiovascular, cerebrovascular, venous thrombosis, longevity, neural tube defects, pregnancy/eclampsia, diabetes, cancer and psychiatry. The T allele is distributed widely among populations showing a high heterogeneity in the prevalence. The frequency is high among Europeans 44% whereas it is within the range from 0 to 94% among Africans. In some Mexican native populations, the T allele frequency is: Tarahumaras 36%, Huicholes 56%, Purpechas 57%, and mestizos 44%. For Yucatecan and Mayan native populations, this information is unknown. It is relevant to know how the C677T allele is distributed in these populations, which have high prevalence of newborn with neural tube defects and cardiovascular diseases. The purpose of this study was to investigate the frequency of the C677T MTHFR mutation between the Yucatecan and Mayan native populations. The C677T mutation was analysed by PCR and the amplified fragments of 198 bp were digested with HinfI restriction enzyme from ADN of Yucatecan healthy volunteers (n=76) and healthy Mayan natives (n=73). Results show that the genotypic frequencies in the Yucatecan population were 29% (22/76) of homozygotes TT, 50% (38/76) of heterozygotes CT and 21% (16/76) of homozygotes CC. For Mayan native population, genotypic frequencies were 41% (30/73) (TT), 45% (33/73) (CT) and 14% (10/73) (CC). These frequencies were in accordance to Hardy-Weinberg expectations in both populations ( $p > 0.33$ ). The allelic frequency was 54% for Yucatecans and 64% for Mayans. Significant differences were not found among both groups ( $p = 0.23$ ). The obtained C677T allele frequencies in the studied populations are high compared with other non-Mexican-populations ( $p < 0.01$ ). The high allelic frequency in these populations might be considered in order to determine risks for having associated diseases with folate metabolism.

**Sequence variation and linkage disequilibrium in the human genome.** *L.M. Frisse<sup>1</sup>, A. Bartoszewicz<sup>1</sup>, J.D. Wall<sup>2</sup>, R.R. Hudson<sup>2</sup>, A. Di Rienzo<sup>1</sup>*. 1) Human Genetics, University of Chicago, Chicago, IL; 2) Ecology & Evolution, University of Chicago, Chicago, IL.

The rate at which linkage disequilibrium (LD) decays with physical distance in human populations has been the subject of considerable debate. Computer simulations and empirical studies have resulted in estimates of LD that extend only a few kilobases from a common SNP, while other studies have documented LD over stretches greater than 100 kb. In order to characterize the effects of population history on LD, we have analyzed ten independent 10 kb non-coding regions in three populations, Italian (European), Hausa, (African), and Chinese (Asian). For each region, a 1-2 kb segment at each end was amplified and sequenced resulting in a set of "locus pairs". By sequencing only the ends of each region, we were able to sample a larger number of independent genomic regions. While re-sequencing every individual in the sample may reduce the number of individuals we are able to examine, it eliminates ascertainment bias from the sample. In addition, it allows the comparison of polymorphism levels, frequency spectrum and LD levels for the same genomic regions and population samples. The results of this survey show that LD decays at different rates in different ethnic groups. In the African sample, the rate of decay of LD with distance is consistent with a simple equilibrium neutral model with effective population size of approximately 10,000 and average recombination rate. However, the decay of LD is much slower in both non-African samples. This observation may be due to the different demographic history of these groups. We also investigate the role of gene conversion to LD decay. We show that the observed pattern of LD is consistent with substantial levels of gene conversion. This leads to the expectation that LD decay over short distances is more rapid than previously thought based on models that included only cross-over.

**Genetic diversity and population structure of two historically related Latin-American populations.** *L.G. Carvajal-Carmona*<sup>1,2</sup>, *R. Ophoff*<sup>3</sup>, *S. Service*<sup>3</sup>, *I. Soto*<sup>2</sup>, *J. Hartiala*<sup>3</sup>, *G. Bedoya*<sup>2</sup>, *J. Ospina*<sup>4</sup>, *V. Reus*<sup>5</sup>, *N.B. Freimer*<sup>3</sup>, *A. Ruiz-Linares*<sup>1,2</sup>. 1) Galton Laboratory, Dept of Biology, University College London, London, England; 2) Laboratorio de Genetica Molecular, Facultad de Medicina, Universidad de Antioquia, Colombia; 3) Center for Neurobehavioral Genetics, School of Medicine, UCLA, USA; 4) Departamento de Psiquiatria, Facultad de Medicina, Universidad de Antioquia, Colombia; 5) Department of Psychiatry, School of Medicine, UCSF, USA.

We are conducting genetic mapping studies in the populations of the Central Valley of Costa Rica (CVCR) and of Antioquia (Colombia). These two populations were established in the 16-17th centuries mostly by the admixture of a small number of Spanish and Native Americans founders. Since their establishment these two populations have remained relatively isolated and have expanded over a thousand times (to a current size of about 3-4 million). Most of the Spanish founders of both areas originated in Southern Spain while the majority of the female founders were from Amerind populations most likely of the Chibchan-Paezan linguistic family (common in lower Central America and North-West South America). To examine the genetic relatedness of the populations currently living in the CVCR and in Antioquia we compared data for 4 mtDNA RFLPs, 10 Y-chromosome polymorphisms and 11 autosomal microsatellites. Both populations exhibited similar levels of genetic diversity with no significant genetic differentiation between them at any of the markers tested. The frequency of Native American haplotypes was similar both for the Y chromosome (CVCR: 5%, Antioquia: 1%) and for mtDNA (CVCR: 83%, Antioquia: 90%), with the mtDNA haplotype distribution being not significantly different from Chibchan-Paezan populations. The similar demographic history and genetic makeup of CVCR and Antioquia should facilitate comparative disease gene mapping in samples from these populations, particularly through the use of LD approaches.

**Assessment of Stratification in Linkage and Association Samples.** *N.C. Martin, S.J. Broxholme, L.R. Cardon.* Bioinformatics, Wellcome Trust Centre for Human Genetics, Oxford, England, United Kingdom.

Population stratification is often cited as a primary contributor of inflated false positive rates in genetic association studies. As a result, a number of robust family-based association strategies have been devised. These methods can protect against inflated Type I error due to sample mixing, but are also very inefficient in their usage of genotype data. An alternative to this strategy has been developed in the form of genomic control (J.K. Pritchard and N.A. Rosenberg, 1999, *Am.J.Hum.Genet.* **65**, 220-228; S.A. Bacanu, B. Devlin, and K. Roeder, 2000, *Am.J.Hum.Genet.* **66**, 1933-1944) in which anonymous genetic markers are used to assess the background degree of stratification and control for it when it is present.

Here we apply the method of Pritchard and Rosenberg to publicly available genome-screen data sets to evaluate the level of stratification in existing samples. The CEPH collection provides an illustrative example for these analyses, comprising more than 30 families that have been genotyped on up to 9,000 genetic markers. Random selection of a set of 40 unlinked markers was conducted 5000 times to evaluate the test statistic distribution. The results indicate a high level of homogeneity in the Utah samples, but increasing heterogeneity in the total sample on addition of the French, Amish and Venezuelan collections. These outcomes show promising sensitivity in the stratification test as a global indicator of sample heterogeneity.

**Eastern African origin of the human maternal lineage cluster, ancestral to people outside of Africa.** *T. Kivisild<sup>1</sup>, M. Reidla<sup>1</sup>, E. Metspalu<sup>1</sup>, J. Parik<sup>1</sup>, T. Geberhiwot<sup>2</sup>, E. Usanga<sup>3</sup>, A. Chaventre<sup>4</sup>*. 1) Estonian Biocentre, Tartu University, Tartu, Estonia; 2) Karolinska University, Stockholm, Sweden; 3) Kuwait University, Kuwait; 4) University Bordeaux 2, France.

More than 95% of non-Africans share a common African maternal ancestor in mtDNA haplogroup L3 via its two sub-clusters M and N. Other sub-clusters of haplogroup L3 are specific to different regions inside Africa. To cast further light on the regional diversification of the African mtDNA pool, in particular to the origin and the spread of haplogroup L3, we have analyzed control and coding region variation in >550 samples from Ethiopia, Egypt and Morocco against the data available for southern and western Africans. Based on complete mtDNA sequences we constructed and rooted the phylogenetic tree relating unambiguously all major African lineage clusters. We also identified a new eastern African mtDNA clade, branching between the roots of haplogroups L2 and L3. This evidence together with a high sequence variation in other sub-clusters of L3 suggests an eastern African origin of haplogroup L3. Phylogeographic founder analysis and coalescent calculations allude to a breakdown of the regional isolation in Africa from the start of the Holocene period.



**DNA polymorphisms of 127 genes on chromosome 21 in Chinese populations.** *W. Huang<sup>1,2</sup>, J.X. Shi<sup>1</sup>, H.F. Xi<sup>3</sup>, Z.W. Jiang<sup>1,3</sup>, K.X. Zhang<sup>1,2</sup>, Y. Wang<sup>1</sup>, L. Jin<sup>1,3</sup>, Z. Chen<sup>1,2</sup>.* 1) Chinese National Human Genome Center at Shanghai, Shanghai, China; 2) Rui Jin Hospital, Shanghai Second Medical University, Shanghai, China; 3) The Institute of Genetics, Fudan University, Shanghai, China.

Single-nucleotide polymorphisms (SNPs) are the most abundant form of genetic variations and have a great potential for mapping genes underlying complex genetic traits. The ultimate value of SNPs for linkage and association mapping depends in part on the allele frequencies of the markers and inter-marker linkage disequilibrium across populations. Although a great deal of effort has been invested in the identification of SNPs, limited information is available of the SNPs including their distribution and allele frequencies on the worldwide populations. As an initial step toward construction of an SNP database of the Chinese populations, we studied the SNPs in 127 genes with known function on chromosome 21 in 30 Chinese individuals derived from different populations along with European and African samples as references. Three chimpanzee samples were also included in this study. Among 60 genes (spanning 318,000 bp) that have been fully analyzed, we identified 549 SNPs. Transition occurred 2.7 times more frequently than transversion. There are 136 SNPs in coding regions, among which, 76 SNPs (55.9%) are non-synonymous. Three genes (AIRE, SOD1 and PRKCBP2) showed deviation from the expectation of neutrality. For 10-20% of the SNPs, their allele frequencies vary substantially among different Chinese ethnic groups and more so with the world populations. 8.9% of the SNPs are specific to Chinese populations and 17.9% can not be found in one of the two reference populations (European and African), suggesting the fallacy of relying SNP discovery on 'model' ethnic groups. For inter-species comparison, we identified 790 nucleotide differences between Human and Chimpanzee over a total of 75,249 bp from 20 genes (1.05 %) and some are shared between species. These data provided interesting first glimpse into the pattern of variation across the genomes among different populations, which suggest that the differences between populations should be considered in the selection of SNPs for the study of complex diseases.

**The Heritability of Early Onset Male Pattern Baldness.** *D.R. Nyholt<sup>1</sup>, N.A. Gillespie<sup>1</sup>, A.C. Heath<sup>2</sup>, N.G. Martin<sup>1</sup>.* 1) Genetic Epidemiology Lab, QIMR, Brisbane, Australia; 2) Department of Psychiatry, Washington University School of Medicine, St Louis, Missouri, USA.

Common baldness (androgenetic alopecia, AGA) is the most common form of hair loss in humans. In Caucasians, normal male hair loss, commonly known as "male pattern baldness" (MPB), is noticeable in about 20% of men aged 20, and continues to steadily increase with age, so that a male in his 90's has a 90% chance of having some degree of MPB.

In addition to being among the most common natural conditions that make men self-conscious, recent studies indicate associations of MPB with benign prostatic hyperplasia (BPH), coronary heart disease, hyperinsulinaemia and insulin-resistance-associated disorders such as obesity, hypertension and dislipidaemia, and that MBP is a risk factor for clinical prostate cancer (CaP). Measures of hair loss were obtained in the course of an interview study of a community-based sample of Australian twin individuals. Ratings, in terms of a global description of hair loss using the Hamilton-Norwood scale (a standard classification scheme which has been shown to have good test-retest reliability), were obtained for a total of 476 MZ and 408 DZ male pairs plus 143 MZ and 154 DZ male individuals aged between 25 and 36 years. Homogeneity test results indicated all stages of hair loss represent the same process (dimension). Subsequently, univariate genetic analysis assumed a single liability dimension (SLD) threshold model. Genetic analysis of the raw categorical data scores using Mx revealed that an additive genetic and non-shared environmental (AE) model best explained individual differences in MPB and that 81% of the total variance could be attributed to additive genetic effects (i.e., a heritability of 81%). Our current sample includes 81 DZ pairs concordant for hair loss, and 132 DZ pairs discordant for hair loss and is an excellent sample in which to pursue quantitative trait locus (QTL) linkage analysis for major genes influencing MPB.

**Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers.** *H. Jernstrom*<sup>1, 2</sup>, *H.T. Lynch*<sup>3</sup>, *P. Ghadirian*<sup>4</sup>, *B. Weber*<sup>5</sup>, *S.A. Narod*<sup>2</sup>. 1) Department of Oncology, The Jubileum Institute, Lund, Sweden; 2) Centre for Research in Women's Health, SWCHSC, Toronto, ON, Canada; 3) Dept. of Preventive Medicine and Public Health, Crighton University School of Medicine, Omaha NE; 4) Epidemiology Research Unit, Centre Hospitalier de l'Univ. Montreal, PQ, Canada; 5) Depts. of Medicine and Genetics and Abramson Family Cancer Research Inst., Univ. of Pennsylvania, Philadelphia, PA.

Several studies have reported that the risk of breast cancer (BC) decreases with increasing duration of breast-feeding. For most studies, the protective effect is greater for premenopausal than for postmenopausal women. An early age at first full-term pregnancy and increasing parity are associated with reduced risk of BC in the general population. However, among BRCA1 and BRCA2 carriers, pregnancy is associated with an increased risk of early-onset BC. The potential for breast-feeding to reduce the risk of BC in BRCA1 and BRCA2 mutation carriers is currently unknown. To evaluate the influence of breast-feeding on the risk of hereditary BC, we conducted a matched case-control study in women who carry deleterious BRCA1 or BRCA2 mutations. Cases were 411 mutation carriers who been diagnosed with BC, and controls were 411 mutation carriers without a history of BC. Cases and controls were matched for age and parity. Information about pregnancies and breast-feeding practices was derived from a questionnaire administered to the women during the course of genetic counseling. Among BRCA1 mutation carriers, the total duration of breast-feeding was sign. shorter for BC cases than for unaffected controls (6.8 vs 9.2 months;  $P = .002$ ). BRCA1 carriers who had breast-fed for more than one year were 40 % less likely to have BC than those who breast-fed for a shorter period OR 0.59 (95 % CI 0.40-0.87;  $P = .009$ ). A protective trend with total duration of breast-feeding was present ( $P = .02$ ). Among BRCA2 mutation carriers, no protective effect was observed from breast-feeding of any duration. BRCA1 carriers who breast-feed for more than one year have a sign. reduction in their risk of BC. Breast-feeding does not appear to confer protection against BC in BRCA2 mutation carriers.

**SNPSTRs: Inferring human evolutionary history from tightly-linked autosomal polymorphisms.** *M.J. Jobin<sup>1</sup>, P. Underhill<sup>2</sup>, A. Lin<sup>2</sup>, A. Knight<sup>1</sup>, L.L. Cavalli-Sforza<sup>2</sup>, J.L. Mountain<sup>1,2</sup>.* 1) Department of Anthropological Sciences, Stanford University, Stanford, CA; 2) Department of Genetics, Stanford University, Stanford, CA.

The mitochondrial genome and the non-recombining portion of the Y chromosome have provided a great deal of information regarding human history in part because there is no recombination within these segments. To extract additional information encoded within the autosomal regions of the nuclear genome, we have developed an approach to the assessment of histories of short autosomal DNA segments. Each such segment includes one short tandem repeat (STR) locus and at least one single nucleotide polymorphism (SNP). We call the combination of an SNP and STR a SNPSTR, and have developed a method for empirically determining phase for double (or multiple) heterozygotes. The SNPs and STR are chosen to be within a degree of proximity that allows recombination to be set to zero, so that the system may be assumed to have a single history. Because the STR and SNPs have dramatically different mutation rates, they provide information of very different resolution. Here we introduce the data derived from the SNPSTR systems we have developed thus far, including the global distribution of the haplotypes of SNPSTR systems on chromosomes 22 and 9. While even a single system sheds light on human evolutionary history, the power of the approach lies in combining the results from multiple SNPSTR systems.

**First author name correction: Sun Ha Jee.**

Program Nr: 1391

**Genetic contribution to waist circumference, hip circumference and waist-to-hip ratio in a lean population: the Korean Cardiovascular Genome Study.** *S.H. Jee<sup>1,2</sup>, H.Y. Park<sup>1</sup>, J.H. Kwon<sup>1</sup>, E.K. Im<sup>1</sup>, E.Y. Cho<sup>1</sup>, J.H. Lee<sup>1</sup>, Y.S. Jang<sup>1</sup>.* 1) Cardiovascular Genome Center, seoul, south Korea; 2) Graduate School of Health Science and Management.

A major gene for abdominal visceral fat area was found in several studies conducted in Western population. Some studies, however, have found after controlling for total fat mass that this major genetic effect was not as strong. To address this issue, we investigated environmental, familial, and genetic influences on waist circumference (WC), hip circumference (HC) and waist-to-hip ratio (WHR) before and after controlling for total fat mass, in 640 members of 78 families who had participated in Korean Cardiovascular Genome Study. Maximum likelihood methods were used to fit several genetic and nongenetic models of inheritance to these data to determine whether an unobserved Mendelian major gene could explain the familial distribution of WC, HC and WHR. Adjustments for age, age<sup>2</sup>, body mass index, smoking, alcohol consumption, and exercise were carried out separately for males and females by multiple regression procedures for WC, HC and WHR phenotypes prior to segregation analysis. WHR was normal distributed and ranged from 0.67 to 1.36, with a mean of 0.84 (male, 0.82 and females, 0.87). WC ranged from 55.5 to 122.8 cm in the entire sample. Correlations between WHR and WC decreased slightly after adjustment for covariates, but remained highly significant. Regression models were used to test genetic and non-genetic models in these 78 families. Segregation analysis did not provide statistical evidence of a major gene controlling either HC or WHR. Mendelian single-locus models with two underlying genotypic distributions were best supported by these data on WC, even after controlling for total fat mass, and this putative major gene explained the 25.8% of variance in adjusted WC. Future linkage studies may be worthwhile to further clarify the mechanisms controlling WC.

**Association between polymorphisms in TNF alfa and beta loci and 9 STR's within HLA region and R/S to Visceral Leishmaniosis in a Colombian population.** *N. Mesa*<sup>1</sup>, *P. Granda*<sup>1</sup>, *AV. Valencia*<sup>1</sup>, *ID. Velez*<sup>3</sup>, *S. Agudelo*<sup>3</sup>, *G. Bedoya*<sup>1,2</sup>, *A. Ruiz-Linares*<sup>1,4</sup>. 1) Molecular Genetics Group, University of Antioquia, Medellin, Antioquia, Colombia; 2) Departamento de Biología, Facultad de Ciencias Exactas y Naturales; 3) Programa para el estudio y control de enfermedades tropicales (PECET). University of Antioquia; 4) Galton Laboratory, University College London.

Leishmaniosis is a parasitic disease produced by hemoflagelated parasite, of which two clinical forms are recognized: the visceral Leishmaniosis (LV) caused by species of the complex *L. donovani* and the cutaneous Leishmaniosis (LC) caused by different species of *Leishmania*. LV is characterized by recurrent chronic fever, esplenomegalie, pancitopenic, weight loss and a high mortality. Segregation analysis has indicated that some genetic determinant could exist with a bigger effect in the susceptibility to the Leishmaniosis and its clinical progression. We evaluated by means of a study of cases and controls, the association grade between resistance/susceptibility (R/S) to the LV and genetics markers (STR's) in HLA region and well as polymorphism in TNF alfa and beta loci. For which, the individual cases were selected with infection antecedents, located in a LV focus from the Caribbean Colombian Coast. The control group with positive Montenegro test, but with out LV an antecedent was paired to the cases by sex, age and exposition risk. Genotipification was done by PCR-RFLP's for loci TNF alfa and beta and 9 STR's in HLA clusters genes (chromosome 6) were resolved using ABI 310 Genetic Analysis. Neither allelic nor genotypic frequencies exhibited significant differences when comparing TNF alfa and beta loci in controls and cases ( $P= 0.5$ ). In spite of the two groups did not show significant genetic structuration, ( $F_{st} = -0.0029$ ), significant differences with respect to linkage disequilibrium for some loci pairs appeared when comparing cases and control groups. We expect to continue evaluating other genomic regions that could be involved in R/S to LV and to elucidate the role of ethnic component in genetic etiology for this disease hence this population have an Amerindian filiation. Grant 9809 University of Antioquia.

**Effects of Population Substructure on the Homozygosity Test of Neutrality under the Stepwise Mutation Model.**

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The composite parameter,  $q = 4Nm$ , where  $N$  is the effective population size and  $m$  is the mutation rate, is an important parameter explaining the statistical properties of genetic variation. Thus, the differences of estimates of  $q$  based on alternative summary measures of variation have been used to develop test statistics to detect natural selection, presence of population substructure, and signatures of past demographic history of populations. In this investigation we show that these test procedures cannot be uncritically used to analyze data on microsatellite loci. Based on analytical as well as simulation studies of coalescent theory, we show that for microsatellite loci evolving via a stepwise mutation model, the number of segregating allele ( $k$ ) is not a sufficient statistic for  $q$ . Consequently, the relationship of expected homozygosity ( $F$ ) and number of segregation alleles ( $k$ ) is different as  $q$  changes. Furthermore, presence of population substructure differentially impacts the relationship of  $F$  and  $k$ . For small  $q$  (say,  $q < 1.0$ ), substructuring generates an excess of  $F$  for a given  $k$ , while the relationship is reversed (i.e., deficiency of  $F$ ) when  $q$  becomes large, particularly when  $k$  is large. As a consequence, the assertion that population substructure mimics the effect of overdominant selection appears to be true only for microsatellite loci with comparatively higher mutation rates and/or in populations of large effective size. (Research supported by NIH grants GM 41399 and GM 58545).

**Different maternal contribution to genetic structures of ethnic populations from Xinjiang Province in China.** *Y-G. Yao, Y-P. Zhang.* Lab. Cellular & Molec. Evol., Kunming Inst. Zool., CAS, Kunming, Yunnan, People's Republic of China.

Previous studies have shown that there were extensive genetic admixture in the Silk Road region. In the present study, we analyzed mtDNA data of 218 individuals from 5 ethnic populations from Xinjiang Province, China, which has been the via route of the Silk Road. In a simple way, we just classified the haplotypes identified into different haplogroups that have been described elsewhere and computed their relative frequencies, to show whether there are any differences in the genetic structure of these populations that performed various cultures. A decreasing frequency of the European-specific haplogroup was observed in these populations, with the highest value present in Uygur (42.6%), followed by Kazak (33.3%), Mongolian (20.4%), and Hui (6.7%), while no European types was found in Han population. Even in the frequencies of the Asian haplogroups, a clear difference was observed among these populations. Combined with the historical records and culture of these populations, recent migration, exogamous, and different extent of cultural isolation might be possible explanations for the observed various maternal contributions to the genetic structure in these ethnic populations.



**Genetic risk factors for cardiovascular disease and hypertension in high-altitude and low-altitude Native American populations.** *J.L. Rupert<sup>1</sup>, K. Kidd<sup>2</sup>, L.E. Norman<sup>3</sup>, M.V. Monsalve<sup>1</sup>, P.W. Hochachka<sup>4</sup>, D.V. Devine<sup>1</sup>.* 1) Pathology and Laboratory Med., Univ British Columbia, Vancouver, BC, Canada; 2) Department of Genetics, Yale University, CT; 3) Stanley Humphries Secondary School, Castlegar, BC, Canada; 4) Department of Zoology, Univ British Columbia, Vancouver, BC, Canada.

There is evidence suggesting that cardiovascular disease (CVD) and hypertension (HT) are less common in high-altitude populations than in those living at sea level. This may reflect increased cardiovascular efficiency associated with adaptive responses to prolonged hypoxia exposure. There are numerous genes with alleles that have been associated with increased risk for CVD and HT. We hypothesised that, over the hundreds of generations that humans have lived in the Andes, selection favouring adaptive cardiovascular fitness may have acted to eliminate these alleles from the gene-pool. To test this hypothesis, we determined allele frequencies for four polymorphisms in the renin-angiotensin system and three polymorphisms in the coagulation cascade in Quechua, an indigenous Andean population, and two lowland populations: Mayans from the Campeche State of the Yucatan and Caucasians of Western European descent. The polymorphisms examined were the insertion/deletion polymorphism in intron 16 of the angiotensin converting enzyme gene (ACE I/D), the C/A1166 mutation in the untranslated 3' region of the angiotensin 2 receptor (type 1) gene, an intronic substitution in the renin gene (G/AI9-83) and the Met235Thr polymorphism in angiotensinogen (AGT); as well as the Arg353Gln polymorphism in Factor VII, the Val34Leu polymorphism in factor XIII and the 5' A/G transition at base -854 in the b-fibrinogen gene. In all cases, allele frequencies were in Hardy-Weinberg equilibrium and, although allele frequencies in the Amerindians differed from those in the Caucasians at several loci (ACE, AGT and b-fibrinogen -854), there were no significant differences in frequency between the two Native American populations. These data suggest that selection has not favoured transmission of alleles at these loci in the Quechua, a high-altitude native population.

**Application of log-linear model of inference on disease susceptibility gene effects under independence of genotype and age.** *N. Tanaka<sup>1</sup>, T. Kinoshita<sup>2</sup>, T. Asada<sup>3</sup>, Y. Ohashi<sup>1</sup>*. 1) Dept Biostatistics, Univ Tokyo, Tokyo, Japan; 2) Dept psychiatry, National center of neurology and psychiatry, Tokyo, Japan; 3) Dept Psychiatry, Inst Clinical Medicine, Univ Tsukuba, Ibaraki, Japan.

Case-control studies provide a powerful approach for detecting disease susceptibility genes or markers that are in linkage disequilibrium with such loci. We focus particularly on situations wherein the genes of interest play roles in several diseases, and allele frequency among disease-free subjects consequently decrease with age. One approach frequently used for analyzing case-control data is logistic model, however, it cannot accommodate the dependence of genotype and age. Using log-linear-models we propose a hierarchical procedure providing a valid method for assessing the interaction in such situation. To assess the bias due to occurrence of other diseases, a theoretical calculation is presented. Then a proposed procedure is applied to simulated data and the observed data of Alzheimer disease and ApoE. By using our procedure, an appropriate inference on genetic effects can be derived whether the interaction is a gene-age interaction or merely a bias which due to occurrence of other diseases.

**Score statistics for linkage based on preferential transmission: when are they valid tests of association? J.**

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Tests for linkage based on the preferential transmission of a particular marker allele to affected children can have considerably more power to detect linkage for complex diseases than traditional sib-pair or LOD-score methods. If such tests are also valid tests of association, then they have the additional property that positive results are suggestive of tight linkage between the disease gene and the marker, and can therefore be useful in localising disease genes.

Score statistics based on preferential transmission for a bi-allelic marker have the general form

$$(N_1 - N_2)^2 / \text{var}(N_1 - N_2) \quad (1)$$

where  $N_i$  is a function of the data counts corresponding to preferential transmission of marker allele  $A_i$ , and  $\text{var}(N_1 - N_2)$  is the variance, or an estimate thereof. The first statistic of this kind to be devised was the TDT. Various statistics of the form of (1) have since been devised for different types of nuclear families, and recently, for general pedigrees also (Martin et al, 2000).

In deriving a statistic of the form (1) to test for linkage, there are often a number of alternatives for  $\text{Var}(N_1 - N_2)$  which may be used. What we show is that this choice is the deciding feature in determining whether or not statistics of this form are valid tests of association as well as linkage. The TDT is a valid test of association as well as linkage for nuclear families with one affected child only. However, our results show that if the variance used in the TDT is replaced by a different variance estimate, then the resulting statistic is a valid test of *both* linkage *and* association when used with nuclear families with any number of affected children and with affected members of larger pedigrees also.

**Regional microsatellite variation in Finland.** *E.T. Salmela*<sup>1,2</sup>, *P. Lahermo*<sup>1</sup>, *M-L. Savontaus*<sup>3</sup>, *P. Sistonen*<sup>4</sup>, *J. Kere*<sup>1</sup>.  
1) Finnish Genome Center, University of Helsinki; 2) Department of Genetics, University of Turku; 3) Department of Medical Genetics, University of Turku; 4) Finnish Red Cross Blood Transfusion Center.

Even though the relationships between the Finns and other European populations have been subject to study and numerous disease gene studies have been successfully conducted among the Finns, the population structure of Finland has received little attention. We measured variation in the allele frequencies of 31 microsatellite markers from 15 different chromosomes among the Finnish population. The selection of the markers was based on the occurrence of rare alleles in the Finnish population observed during the routine genome wide scans performed at the Finnish Genome Center. Blood samples of 465 males were collected from different parts of Finland and grouped into nine subpopulations based on the birthplaces of their grandparents. The purpose of the study was to measure the quality and quantity of genetic variation within and between different regions of Finland. The earlier observations based on blood group markers have suggested substantial differences in the allele frequencies of individual markers on the community level but more subtle ones on the county level, and they were supported in this analysis. The total amount of variation was not conspicuously different between the regions of late and early settlement, although differences in the amount of variation between the individual counties within these two groups could be seen. No clear East-West cline was observable in the initial analysis. North Carelians in the east and Swedish-speaking Ostrobothnians in the west had the largest amount of deviations from the average allele frequencies of the Finns. These observed trends are expectable with regard to the Finnish population history.

**The origins of the Negroid Makrani population from Pakistan: maternal and paternal perspectives.** *L. Quintana-Murci<sup>1</sup>, R. Qamar<sup>2</sup>, S.Q. Mehdi<sup>2</sup>, Q. Ayub<sup>2</sup>, A. Mohyuddin<sup>2</sup>, T. Zerjal<sup>4</sup>, H.J. Bandelt<sup>3</sup>, K. McElreavey<sup>1</sup>, C. Tyler-Smith<sup>4</sup>.*  
1) Dept Human Immunogenetics, Pasteur Inst, Paris, France; 2) Biomedical & Genetic Engineering Division, Islamabad, Pakistan; 3) University of Hamburg, Germany; 4) Department of Biochemistry, University of Oxford, UK.

The Negroid Makrani population live near the Makran Coastal range in south-western Pakistan and are considered to have strong African roots based on anthropological and cultural evidence. The origins and affinities of this population have been investigated through a detailed genealogical study of mitochondrial DNA (mtDNA) and Y chromosome variation. RFLP and control region (CR) sequence analysis revealed that more than 40% of the Makrani mtDNA lineages belong to sub-Saharan African lineages, including L1a, L2, L3b and L3d. These lineages were not observed in more than 300 individuals belonging to other Pakistani ethnic groups, including Hazara, Hunza, Brahui, Parsi, Pathans, Balouchi, Sindhi and Kalash Kafirs. The Eurasian haplogroup U is the second most represented lineage (25%) in this population, the most dominant subcluster being the ancient U2 lineage. Haplogroup M, which has been proposed to trace an ancient migratory coastal pathway from East-Africa to India, was found at only 6% in the Negroid Makrani, but at more than 30% in the other coastal populations. The proportion of typical western-Eurasian lineages (H, I, J, T, X) in this population is notably lower than in the other Pakistani groups. In sharp contrast, the Y chromosome haplogroup distribution in the Negroid Makrani population is similar to the other Pakistani populations and distinct from African populations. The most represented lineages are haplogroups 3 (30%), 9 (18%) and 1 (18%). In conclusion, the study of the maternal and paternal gene pool of the present-day Negroid Makrani reflects that the sub-Saharan African contribution is mainly of matrilineal origin and that the African male-mediated gene flow has been very limited. Together, these results are consistent with significant directional mating between local males and females related to Bantu-speaking populations from sub-Saharan Africa.

**Genetical diversity of two isolated African-Brazilian populations.** *K.A. Sandes<sup>1</sup>, W.A. Silva Jr<sup>2,3</sup>, S.M.B. Sousa<sup>1,4</sup>, A.A.L. Barbosa<sup>1</sup>, P.L. Santos<sup>1</sup>, M.O.Q. Santos<sup>1</sup>, P.R.V. Bastos<sup>1</sup>, E.S. Souza<sup>1</sup>, M.A. Zago<sup>2,4</sup>.* 1) Universidade Estadual do Sudoeste da Bahia-Jequie-UESB; 2) Centro de Terapia Celular - Hemocentro de Ribeirao Preto; 3) Universidade Federal do Para - Nucleo Santarem; 4) Faculdade de Medicina de Ribeirao Preto - Universidade de Sao Paulo, Brazil.

Barra and Sao Goncalo are two communities located in Bahia, a State in the northeast region of Brazil. Both of them are constituted by black individuals who are descendent of ancient slaves brought from Africa. These communities are considered isolated in reproductive and cultural aspects, since they show high levels of endogamy and low migration levels. With the purpose of studying the genetical diversity of these isolated communities, we have analyzed the mitochondrial DNA hypervariable segment (HVS I) and have compared the sequences with the reported data from Caucasian, African and Amerindian populations. This segment from 220 individuals was amplified by PCR and sequenced in an ABI377™ automated sequencer. Thus far, 127 sequences have been analyzed and 56 different haplotypes have been identified. On the basis of the "Cambridge reference sequence" (Andrews et al., 1999), 93 variable sites were observed, 67 (72%) of them being polymorphic. The 16,223 (C®T) polymorphic site was the most frequent both in Barra and in Sao Goncalo (96%), a polymorphism reported to be found in 91% of typical African lineages. In average, 7% of the studied lineages were classified as being Amerindian. The FST values show that only 13.25% of the total genetic variation are attributed to differentiation between the two communities, which means that 86.75% correspond to the variability within each population. Although the two communities have similar origins and history, they do not share any common sequence.

**Founder effect in North-Eastern Quebec and the extent of the genetic signature associated with the myotonic dystrophy mutation.** V. Yotova<sup>1</sup>, E. Zietkiewicz<sup>1</sup>, E. Lemieux-Blanchard<sup>1</sup>, M. Labuda<sup>1</sup>, S. Bourgeois<sup>1</sup>, D. Labuda<sup>1</sup>, J. Fortin<sup>2</sup>, P. Lepage<sup>2</sup>, T.J. Hudson<sup>2</sup>, A. Lescault<sup>3</sup>, C. Laberge<sup>3</sup>. 1) Research Center, Hopital Sainte-Justine, Montreal, Quebec, Canada; 2) MGH Research Center, McGill University, Montreal Genome Center, Montreal, Quebec, Canada; 3) Centre hospitalier, Universit Laval, Ste-Foy, Quebec, Canada.

Myotonic dystrophy is frequent in French-Canadian population of North-Eastern Quebec (1:650). This dominant disorder is due to a CTG-triplet expansion in the DMPK gene on chromosome 19q13 (OMIM160900). Rather than directly analyzing the expansion, we wanted to define a unique haplotype composed of single-nucleotide polymorphisms, a SNP signature, eventually amenable to automatic screening. By the same token, we investigated the extent of the ancestral carrier-haplotype conservation in a young population issued from a founder effect and addressed the hypothesis of a single introduction of the DM-mutation in North-Eastern Quebec. SNPs were found *in silico* as well as experimentally in a population panel by DHPLC and subsequent sequencing. Twenty of these SNPs were typed in 51 DM-families by allele-specific oligonucleotide hybridization. The resulting haplotype extends over 2.3 Mb according to the recent UCSC map. There are two groups of DM haplotypes. The first, presumably young, is represented by a haplotype seen in 34 copies and a minor recombinant (2 copies). The second is represented by four variants (5, 5, 3 and 2 copies) that can be mutually related through single recombinations. These two groups could therefore represent two separate entries of the DM-chromosomes. On the other hand, they share a shorter core haplotype, suggesting a relatively recent common origin for both groups; genealogical data will help to trace it back in time. In conclusion, the DM-mutation appears to be associated with a limited number of unique SNP-signatures that are not shared with non-affected chromosomes; shorter segments of these haplotypes are relatively common in the population; our data demonstrate an important increase in the extent of linkage disequilibrium that can be related to the young age of the population of Quebec. (Supported by RMGA FRSQ).

**Effect of the Her2 V655I polymorphism on breast cancer risk in BRCA1/2 mutation positive and negative families.** *J.L. Rutter, N. Chatterjee, S. Wacholder, J. Struewing.* Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

Overexpression of the Her2 proto-oncogene has been implicated in carcinogenesis of breast cancer, and is associated with poor prognosis. A polymorphism at codon 655 results in a valine to isoleucine change, and the valine allele has been associated with an elevated risk of breast cancer. We asked whether this Her2 V655I polymorphism could be an important susceptibility biomarker for breast cancer risk in women with and without BRCA1/2 mutations. We have a community-based collection of DNA samples and personal and family cancer history data on 5,318 Ashkenazim volunteers from the Washington, DC area. We extended the kin-cohort method to estimate age-specific breast cancer penetrance by Her2 variant status and also by the joint status of Her2 and BRCA1/2 mutations. Kin-cohort analysis uses the disease history of the relatives of the genotyped volunteers to estimate risk, and the volunteer's genotype information to infer a probabilistic distribution of the possible genotypes among the individual relatives. A subset of 769 subjects was identified that maximizes the information content with the fewest number of assays: 116 were BRCA1/2 mutation carriers, 454 were breast or ovarian cancer survivors or had two first degree relatives with breast cancer or a single relative with breast cancer before the age of 50, and 199 subjects were randomly selected. Her2 genotype frequencies for these 769 volunteers were 74% I/I, 23% I/V, and 2% V/V. Cumulative risks of breast cancer up to age 50, 60, and 70 were calculated. Breast cancer risk was significantly elevated for the BRCA negative/Her2 valine positive subjects, with relative risks (RRs) of 1.71, (95% CI 1.21, 5.76), RR = 1.40 (CI = 1.15, 4.19), and RR = 1.40 (CI = 1.18, 3.32) in the three age groups, respectively. Age-specific relative risks were similar among BRCA1/2 positive subjects, although these did not reach statistical significance due to low numbers. These results suggest that the risk of breast cancer is increased in carriers of the Her2 valine allele, and that the risk may be greater in younger women.



**The coalescence times of human genes and the divergence times of human populations.** *N.A. Rosenberg, M.W. Feldman.* Department of Biological Sciences, Stanford University, Stanford, CA.

The *divergence time* of two populations is the length of time that has elapsed since the populations arose from an ancestral group, while the *coalescence time* of a set of copies of a gene is the length of time since the most recent common ancestor of the gene copies was alive. If no migration has occurred between two descendant populations, the coalescence time of a given locus for a sample taken from the two populations must predate the divergence time of the populations. If migration has occurred, however, the coalescence time may be more recent than the divergence time. Using analytical results and simulations, we describe the relationship between coalescence times and divergence times in population genetic models with and without migration. Two main parameters impact this relationship: the ratio of the divergence time in generations to the size of each population ( $TD/N$ ) and the total number of migrants entering each population during each generation ( $Nm$ ). For small values of  $TD/N$ , the coalescence time exceeds the divergence time by a proportionately large amount. For large values of  $TD/N$  and small values of  $Nm$ , this excess is fairly small. If both  $TD/N$  and  $Nm$  are large, the probability that coalescence is more recent than divergence approaches 1. A bimodal distribution of coalescence times across loci is a signature of an intermediate amount of migration between descendant populations.

Selecting reasonable values for  $TD/N$  and  $Nm$ , the ancient coalescence times of certain human genes are seen to lie well within the predicted distribution of coalescence times under a recent origin of modern humans from an African founder population. These coalescence times are also consistent with a more ancient origin followed by migration among descendant populations. Additionally, the relatively ancient coalescences of Native American lineages need not contradict a relatively recent entry of humans into the New World. Genome-wide distributions of coalescence times from different subsets of the global population should soon become useful for testing hypotheses about the divergence times of human populations.

**ELAC2(HPC2) variation does not contribute to elevated prostate cancer risk in the Afro-Caribbean population of Tobago.** *P.R. Shea<sup>1</sup>, R.E. Ferrell<sup>1</sup>, A.L. Patrick<sup>2,3</sup>, L.H. Kuller<sup>2</sup>, C.H. Bunker<sup>2</sup>.* 1) Departments of Human Genetics and; 2) Epidemiology, University of Pittsburgh, Pittsburgh, PA; 3) Tobago Regional Health Authority, Trinidad and Tobago.

Recently, ELAC2 was identified as a positional candidate gene for prostate cancer (PCa) susceptibility. Two common missense variants, Ser217Leu and Ala541Thr, were found to be associated with familial and sporadic PCa, particularly among cases diagnosed prior to age 60. Previous studies have included a limited number of individuals of African ancestry. Afro-Caribbean males from Tobago have an elevated screening-detected prevalence of PCa (135/1000 in men over 50 years of age). As part of a systematic attempt to identify genes that influence the risk of PCa in the Tobago population, we screened 93 cases and 196 controls for the ELAC2 217L and 541T alleles reported to increase risk of PCa. The Tobago population was found to be homozygous for the common A541 (low-risk) allele in all cases and controls. The frequency of the 541T allele in African Americans was previously reported to be 0.8% compared to 3.2% in Caucasians. The proportion of the 541T allele in African Americans suggests that it is a Caucasian allele present in African Americans due to admixture. This is consistent with historical data suggesting that there is little non-African admixture in the Tobago Afro-Caribbean population. The frequency of the "high-risk" 217L allele in the Tobago cases was 0.21 compared to 0.23 in controls. In order to eliminate the possibility of African-specific alleles in ELAC2, we sequenced the entire coding region of ELAC2 in 24 Tobago prostate cancer patients and did not observe any additional variation. These data suggest that the PCa susceptibility gene, ELAC2, identified in Caucasians does not contribute to the elevated risk of PCa in the Tobago population.

**Fecundity is a familial trait in the Old Order Amish.** *T.I. Pollin<sup>1</sup>, R. Agarwala<sup>2</sup>, A.A. Schäffer<sup>2</sup>, A.L. Lodge<sup>1</sup>, T.M. King<sup>3</sup>, A.R. Shuldiner<sup>1</sup>, B.D. Mitchell<sup>1</sup>.* 1) University of Maryland, Baltimore, MD; 2) National Center for Biotechnology Information, NIH, Bethesda, MD; 3) University of Texas, Houston, TX.

Few studies have examined the genetic influences on fecundity in human populations. The Old Order Amish may be useful for this purpose because their religious beliefs strongly encourage large families and prohibit the use of contraception, and they are a closed founder population with well-documented genealogies dating back to the 18th century. From a large Amish genealogical database, we selected for analysis of fecundity the 2246 members who (1) were descendants of one or both of the genealogy's founders, (2) had survived to at least age 60 by the time the genealogy was compiled (1988), (3) had married only once and (4) were married to an individual meeting the first three criteria. These individuals (comprising 1123 couples) had an average of  $7.1 \pm 3.5$  (mean  $\pm$  SD) children, with a range from 0 to 18. Mean number of children did not change over time (maternal birth cohort years 1787-1927; Pearson correlation coefficient = -0.03,  $p = 0.40$ ).

Using quantitative genetic methods, we estimated the heritability of fecundity, considered as a quantitative trait, to be significantly greater than 0 ( $h^2 = 0.15 \pm 0.02$ ;  $p = 3.7 \times 10^{-24}$ ). Seventy-four individuals (37 couples) had no children, indicating a frequency of complete infertility of 3.3%. However, the ratio of the sibling recurrence risk of this trait to the overall risk did not differ appreciably from 1 [ $I_s = 0.74$ ; 95% CI: (0.30, 1.80)].

We conclude that there is significant evidence for familial aggregation of fecundity in this population, although we were unable to detect a significant genetic influence on gross infertility. The observed familial nature of fecundity may result from either familial clustering of behavioral characteristics or alternatively from genetic influences on reproductive biology.

To be presented by J. Sequeiros instead of A. Sousa.

Program Nr: 1406

**Familial amyloid neuropathy (FAP Met30) in Portugal: a combined use of family studies and a 60 years' register in the assessment of anticipation of age-at-onset and of the evolutionary dynamics of the gene.** A. Sousa<sup>1,2,3</sup>, A.M. Silva<sup>2,4</sup>, M. Marta<sup>2,4</sup>, J. Sequeiros<sup>1,3</sup>, T. Coelho<sup>2,5</sup>. 1) Dept Population Studies, ICBAS, Porto, Portugal; 2) Unidade Clinica Paramiloidose, HGSA, Porto, Portugal; 3) UNIGENE, IBMC, Univ. Porto, Portugal; 4) Dept Neurology, HGSA, Porto, Portugal; 5) Dept Neurophysiology, HGSA, Porto, Portugal.

Familial amyloid neuropathy, an AD disease due to a point mutation in chr18q11.2-q12.1, was first described in Portugal by Andrade (1952). Portugal represents the largest focus of the most frequent mutation worldwide (Val30Met). Between 1939 and December 1999, 1599 patients (from 478 independent kindreds) were diagnosed at HGSA/CEP in Porto (Portugal), 925 of which since molecular diagnosis became available (in 1985). Proband's who report absence of disease in either parent have increased. This does not represent de novo mutations but incomplete penetrance in previous generations, as often proven by molecular diagnosis. Mean age-at-onset was significantly higher in patients with no affected parent (45.3 yrs., SD 12.6) than in patients with one affected parent (31.6 yrs, SD 7.1). We studied 428 parent-offspring pairs, where both were affected and observed at the Center in the past 60 years: mean anticipation was 7.1 yr. (SD 10.4), being 9.5 yrs. when the disease was maternally inherited and 4.4 yrs when the transmitter was the father. After excluding pairs involving a proband, mean anticipation was still 6.4 yrs. In order to correct for a cohort effect (excess of offspring with early onset), we also excluded pairs where offspring were born before 1950: mean anticipation was still 6.6 yrs. A random sample of pairs yielded a similar result. These methods of correction for ascertainment point out to a true biological phenomenon. On the other hand, among 221 families diagnosed since 1985, 112 probands (mean age-at-onset: 33.8 yrs) had one affected parent while for the other 109 (mean 48.4) FAP was unknown in previous generations. These two apparent antithetical features (anticipation running in families and probands of new families having later onset) need further investigation in order to understand the evolutionary dynamics of this mutation.

**Postgenome Strategies for Mapping Disease Susceptibility Loci.** *B. Rannala, J.P. Reeve.* Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada.

The imminent availability of a complete annotated human genome sequence (AHGS) necessitates the development of new methods for disease gene mapping. Existing methods assume that no information is available about the position of a disease mutation prior to a marker-based study of affected individuals. However, the distribution of genes across the genome modifies the probabilities that particular regions harbor disease mutations. If combined with an extensive database of single nucleotide polymorphisms (SNPs) or other markers, an AHGS can also influence the choice of genetic markers; different strategies are possible such as choosing markers uniformly over a region, preferentially choosing markers within exons, etc. Information from databases that catalogue the positions of mutations in known disease genes can also be used as a guide in marker choice. New multipoint statistical methods are presented for mapping disease susceptibility mutations using either linkage analysis, or linkage disequilibrium analysis, and taking full account of information from a human mutation database and an AHGS. Monte Carlo computer simulations are used to study the statistical accuracy and power of these methods. The accuracy and power of several strategies for choosing SNPs or other markers, in the context of an AHGS, for use in mapping studies of either simple, or complex, genetic disorders are also examined by computer simulation.

**Geographic latitude and frequency of the G protein b3 subunit (GNb3) C825T variant are strongly associated with blood pressure in a worldwide ecological analysis.** *J.H. Young, W.H.L. Kao, A. Chakravarti, M.A. Levine, M.J. Klag.* Departments of Medicine, Epidemiology and Genetics, Johns Hopkins, Baltimore, MD.

Climatologic factors that vary by latitude influence blood pressure (BP) and may have driven evolution of genes involved in BP regulation. To test this hypothesis, we determined the extent to which latitude and the hypertension candidate variant C825T of GNb3 contribute to inter-population variation in BP. Phenotype data were obtained from the Intersalt Study, a worldwide epidemiologic study of BP in 52 populations. Standardized protocols were used to measure systolic blood pressure (SBP), 24-h-sodium excretion (24-h-Na), body-mass index (BMI), and alcohol intake; population averages of each variable were used for these analyses. Estimates of the GNb3 825T frequency were obtained from previously published studies of this allele in populations that were ethnically similar and geographically approximate to Intersalt populations. Regression analyses were performed using 35 of 52 Intersalt sites with available genotype data. Mean SBP (mmHg) ranged from 96.0 to 127.0 (mean = 117.5). Absolute latitudes ranged from 0.18 to 61.6 degrees and 825T frequencies ranged from 0.21 to 0.85. In univariate analyses, latitudes were associated with mean SBP while 825T frequencies were not ( $P < .001$  and  $P = 0.9$ , respectively). However, after adjusting for latitude, 825T frequencies were strongly and positively associated with mean SBP ( $P < .001$ ). In univariate analysis, latitude explained only 28% of the variation in mean SBP; however, when 825T frequencies were added to the model, these two variables accounted for 65% of the inter-population variation in mean SBP. Adjustment for mean 24-h-Na, BMI or alcohol intake did not change these results significantly. The ecological nature of this analysis limits causal conclusions; 825T frequency may represent other latitude-correlated gene variants that influence BP or a non-genetic aspect of ethnicity related to BP. This study shows that latitude and GNb3 C825T are strong predictors of inter-population variation in SBP. These results suggest that ecological studies may be effective tools for the identification of genes involved in BP regulation.

**Parallels and contrasts in female and male human evolutionary history.** *P. Shen<sup>1</sup>, H. Tang<sup>2</sup>, P. Passarino<sup>5</sup>, B. Do<sup>1</sup>, R. Sung<sup>1</sup>, K. Davis<sup>1</sup>, C. Sharfe<sup>1</sup>, L.L. Cavalli-Sforza<sup>3</sup>, M.W. Feldman<sup>4</sup>, P.J. Oefner<sup>1</sup>.* 1) Genome Technology Ctr; 2) Departments of Statistics; 3) Departments of Genetics; 4) Departments of Biological Science, Stanford Univ., Palo Alto, CA; 5) Department of Cell Biology, University of Calabria, Rende, Italy.

Binary polymorphisms associated with the non-recombining region of the human Y-chromosome (NRY) and mitochondrial DNA preserve the paternal, respectively maternal genetic legacy of our species that has persisted to the present, permitting inference of human evolution, population affinity and demographic history. Using both denaturing high-performance liquid chromatography and dye-terminator sequencing, we screened 108 males from the five continents on 70 kb of NRY sequence, including the genes DFFRY, DBY, UTY1, TB4Y and EIF1AY, and 400 representatives of >50 ethnic groups on 15.6 kb of non D-loop mtDNA sequence. A total of 127 NRY and 1,709 mtDNA variable sequence sites were found, respectively. Based on genetic distance and mismatch distribution analysis a minority of East Africans, Sans and Pygmies represent the descendants of the most ancestral patrilineages of anatomically modern humans and their effective population size appears to have remained constant. Mismatch analysis shows that all other mtDNA and NRY lineages split from this ancestral group ~130,000 and ~72,000 years ago, respectively. There is strong evidence for a major expansion having occurred in mtDNA and NRY effective population size about 50,000 and 33,000 years ago. Mitochondrial DNA data suggest a second population expansion ~12,000 years ago in Europe at about the time of the Neolithic migration into Europe. A tree-partition method that does not depend on the demographic history of the population was used to estimate the time to the most recent common ancestor (TMRCA). The estimated TMRCA for NRY was ~92,000 years, while that for mtDNA was ~230,000 years. The discrepancy between NRY and mtDNA age estimates is the result of a significant reduction in variability on the NRY caused by selection, or demographic differences between males and females that led to the replacement of a substantial portion of the indigenous male pool in Africa.

**Reconstructing the prehistory of Ugandan populations.** *D. Barkhan*<sup>1</sup>, *T. Jenkins*<sup>1</sup>, *W. Byarugaba*<sup>2</sup>, *H. Soodyall*<sup>1</sup>. 1) MRC/Wits/SAIMR Human Genetic Diversity and Disease Research Unit, Dept. of Human Genetics, SAIMR/University of the Witwatersrand, Johannesburg, South Africa; 2) Department of Pathology, Makerere University, Uganda.

We have used mitochondrial DNA (mtDNA) and Y chromosome DNA to examine the gene pool of present day Ugandan populations. Our sample from Western Uganda represents four Bantu-speaking ethnic groups.

*mtDNA studies:* We screened for the presence of the mtDNA 9bp deletion in 259 unrelated individuals and sequenced ~800bp of the mtDNA control region in 89 individuals. The 9bp deletion was found in 18/259 individuals (6.9%). Control region sequencing revealed that 8/11 (72.7%) individuals with the deletion had sequence profiles representing the common form of the deletion in Africa. We derived 72 unique mtDNA types defined by 111 polymorphic sites from pairwise comparisons of sequences. MtDNA types associated with the 9bp deletion clustered in a separate branch in the NJ-tree. The tree could not resolve mtDNA types found in individuals from the different ethnic groups. Using the established mtDNA haplogroup nomenclature, we estimated that 40% of mtDNA types found in Ugandans belong to haplogroup L1, 16% to haplogroup L2 and 44% to haplogroup L3.

*Y chromosome studies:* We used the Y *Alu* insertion polymorphism (YAP) and four short tandem repeats (DYS393, DYS19, DYS390, DYS391) to derive Y chromosome haplotypes in 120 males. We derived 29 unique haplotypes. Three YAP+ haplotypes (YAP+-15-17-21-10, YAP+-14-17-21-10 and YAP+-13-15-21-10) together represented 48% of Ugandan Y chromosomes. We used networks to examine the distribution of Y chromosome haplotypes among the different ethnic groups, and to examine the evolutionary relationships of haplotypes found in Ugandans and neighbouring populations.

We discuss the genetic affinities of Ugandan populations with other sub-Saharan African populations, and evaluate relative female and male contributions to the gene pool of present day Ugandans.



## **Y-CHROMOSOME BIALLELIC POLYMORPHISMS AND NATIVE AMERICAN POPULATION**

**STRUCTURE.** *M. Bortolini*<sup>1,2</sup>, *F. Salzano*<sup>2</sup>, *C. Bau*<sup>2</sup>, *Z. Layrisse*<sup>3</sup>, *M. Petzl-Erler*<sup>4</sup>, *L. Tsuneto*<sup>4</sup>, *K. Hill*<sup>5</sup>, *A. Hurtado*<sup>5</sup>, *D. Castro-de-Guerra*<sup>3</sup>, *G. Bedoya*<sup>6</sup>, *A. Ruiz-Linares*<sup>1,6</sup>. 1) Department of Biology, University College London, London, UK; 2) Departamento de Genetica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil; 3) Departamento de Genetica, UFP, Curitiba, Brazil; 4) IVIC, Caracas, Venezuela; 5) Department of Anthropology, UNM, Albuquerque, USA; 6) Laboratorio de Genetica Molecular, Departamento de Fisiologia y Bioquimica, Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia.

We recently evaluated South Amerind population structure using data from mtDNA RFLPs and Y-chromosome STR and failed to detect a significant difference in migration rates between males and females (Mesa et al., 2001:AJHG; 67: 1277-1288). However, the high mutation rate of STR might affect estimates of genetic structure for the Y-chromosome. To assess the dependence of our conclusions on the type of Y-chromosome marker analyzed we examined 7 Y-biallelic markers in 356 individuals from 17 Native South American populations. Data for most of these markers are also available for a number of native populations from Central and North America. Within and between population diversity was also assessed for these regions using available mtDNA RFLPs data. For both Y-chromosome and mtDNA markers we observe increasing levels of population structure from North to South America with Central America showing intermediate values. This trend is more pronounced for Y-chromosome than for mtDNA, resulting in an inversion of the relative male/female migration rate between North and South America (from about 3/1 to 1/2). These new analyses are consistent with our previous findings and emphasize the importance of genetic drift in the evolution of Native American populations.

**DNA Pooling, Genome Control and Association Studies.** *L. Hill, A. Jawaid, P. Sham, R. Plomin.* SGDP Research Centre, Institute of Psychiatry, King's College London, London, UK.

In case-control studies, ethnic stratification can lead to spurious associations between loci and phenotype. If population samples are well matched, then allele frequencies will tend to be similar between cases and controls for unlinked markers, and will only show significant allele frequency differences when the marker is near susceptibility loci. If stratification is present, however, then more widespread allele frequency differences should be detected. Most association studies to date have only examined candidate regions, containing small numbers of markers. Ethnic stratification cannot be eliminated in these instances, unless additional unlinked markers are genotyped. Pritchard and Rosenberg (*Am J Hum Genet*, 65:220-228, 1999) suggest that, for microsatellites, genotyping an additional 15 - 20 unlinked loci are sufficient to test for stratification.

As part of a genome wide association scan for cognitive ability, we have examined 1847 SSR markers at approximately 2 cM intervals throughout the genome using DNA pooling as a screening tool. Prior to including all 1847 markers, we studied 60 markers on chromosome 1 as a paradigm study, to ascertain that our case-control samples are not subject to stratification. We extended genomic control to DNA pooling using estimated allele frequencies to calculate a Chi squared statistic for each marker, which were then summed to generate the stratification statistic. The genomic control results based on DNA pooling indicated that no stratification was present ( $p = 0.986$ ). To our knowledge, this is the first genome wide association scan to assess stratification using the genome control method with DNA pooling.

**Analysis of population structure of Japanese population using genotype data of genome wide hundreds of SNPs.**

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Linkage disequilibrium mapping is used to locate disease susceptibility loci. This technique, using a dense map of single nucleotide polymorphisms (SNPs), has been advocated as the method of choice for complex genetic diseases. A serious problem with linkage disequilibrium mapping has been suggested that population structure could lead to many spurious associations between genetic markers and a disease phenotype, especially for studies using samples from ethnically heterogeneous region. Japanese population has been considered to be less heterogeneous, and, therefore, more appropriate for linkage disequilibrium mapping than many other populations in the world, because of its ethnic isolation both historically and geographically, although no analysis based on genotype data has been reported. In order to obtain evidence of homogeneity of Japanese population, we investigated population structure of Japanese population with genotype data of hundreds of individuals and of hundreds of unlinked SNPs distributed throughout the genome. We adopted two analytical methods. One method is to assess existence of difference in population structure between two sample groups from geographically different sampling locations in Japan, by analyzing chi square statistics computed for multiple contingency tables constructed for an association test between allele frequency of each SNP and sub-populations. The other is to infer population structure by model-based clustering method using Markov chain Monte Carlo algorithm incorporating genotype data of multiple SNPs. We found that no significant population structure existed between two sample groups, each of which represented one of the two largest Japanese metropolises, Tokyo and Osaka.

**Inbreeding in Eastern Antioquia (Oriente), Colombia.** *I. Soto-Calderon<sup>1</sup>, P. Montoya<sup>1</sup>, J. Ospina<sup>1</sup>, G. Bedoya<sup>1</sup>, A. Ruiz-Linares<sup>1,2</sup>.* 1) Laboratorio de Genetica Molecular, Departamento de Fisiologia y Bioquimica, Facultad de Medicina, Universidad de Antioquia, Medellin, Colombia; 2) The Galton Laboratory, Department of Biology, University College London.

The population of the province of Antioquia in North-West Colombia was established in the 16th-17th century through the admixture of a small number of immigrants (mostly Spanish) and Native Americans. Historical records indicate that the demographic growth of this region occurred in relative isolation and several founder effects for Mendelian disorders have been documented in the province. Some of the oldest Antioquian settlements were established to the East of the province (Oriente region) where a concentration of cases for Mendelian (albinism, cystic fibrosis) and complex (bipolar) disorders has been noted. We surveyed surname frequencies in Antioquian municipalities and found that the Oriente region differed significantly from other areas. The inbreeding coefficient estimated from isonymy in three Oriente municipalities ranged between 0.01 and 0.02 with 7 surnames accounting for about 50% of those present in this area. To examine the temporal trend in inbreeding, information from census records for the years 1667, 1786, 1813, 1843 and 1851 and from marriage and baptismal Church records between 1720 and 1836 was examined for one of the oldest Oriente municipalities (Marinilla). The inbreeding coefficients estimated from surname frequencies varied between 0.006 and 0.008 with a trend towards an increase throughout the 19th century. For the period between 1800-1813 information on consanguineous marriage dispensations resulted in an inbreeding coefficient of 0.002. The level of inbreeding in Oriente as assessed by these two methods is similar to that seen in other human population isolates and indicates favorable conditions for genetic mapping studies in Antioquia.

**GAA triplet-repeat variation at the Friedreich ataxia locus in Nahuatl Indians of Mexico.** *R.M. Clark<sup>1</sup>, S. Bhatti<sup>1</sup>, E. Alonso<sup>2</sup>, P. Yescas<sup>2</sup>, A. Rasmussen<sup>2</sup>, S.I. Bidichandani<sup>1</sup>.* 1) Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Neurogenetics, Instituto Nacional de Neurologia y Neurocirugia Manuel Velasco Suarez, Mexico City, Mexico.

Friedreich ataxia (FRDA) is caused by abnormal expansions of a polymorphic GAA triplet-repeat sequence in the first intron of the frataxin gene. Normal and mutant chromosomes have 6-60 and 100-1700 triplet repeats, respectively. Population studies have shown that the people of Europe, North Africa, Middle East and India have the same distribution of GAA triplet-repeat alleles; 85% alleles contain 6-12 triplets (short normal, "SN"), 10-15% contain 13-60 triplets (long normal, "LN"), and approximately 1% of the chromosomes have pathologic expansions with >100 triplets. Haplotype analyses indicate that expanded alleles arise from a sub-population of LN alleles (those with at least 34 uninterrupted GAA triplets). In fact, people from East Asia (Japan, China, and South East Asia) have a significantly lower frequency of LN alleles (<1%), and consequently have a very low incidence of FRDA. A study of 66 chromosomes from Algonquin speaking Amerindians (Quebec and Ontario) showed a complete lack of LN alleles, apparently supporting an ancestral link with the populations of East Asia. We have conducted a study of a large cohort of another North American Indian population, the Nahuatl Indians of Mexico. Contrary to the data from Algonquins, we identified 7 LN alleles among the 344 Nahuatl chromosomes analyzed (2%). Analysis of 166 non-FRDA chromosomes from an otherwise unselected cohort of Mexican individuals revealed 10 LN alleles (6%), i.e., the Nahuatl population has a significantly lower prevalence of LN alleles ( $P = 0.0014$ ). These data have important public health implications for the incidence of FRDA among the native Indian populations of North America, in addition to the anthropological implications regarding their ancestral origin(s).

**Androgen receptor gene CAG and GGN polymorphic repeats and obesity in older adults.** *D.R. Gustafson, B.M. Koppnati.* Dept Nutrition & Food Sciences, Utah State Univ, Logan, UT.

The genetic basis for obesity and body fat distribution in humans is unclear. The X-linked androgen receptor gene (AR) has two polymorphic trinucleotide repeats in exon 1 related to transcriptional activity of the androgen receptor. Since differences in sex hormone metabolism relate to obesity patterns in men and women, AR variants may relate to obesity. We investigated the association between indices of obesity and AR (CAG)<sub>n</sub> and (GGN)<sub>n</sub> polymorphisms in a cross-sectional study of 103 men (51-93 years) and 117 women (51-92 years). AR (CAG)<sub>n</sub> and (GGN)<sub>n</sub> were quantified using Perkin Elmer Applied Biosystems GeneScan analysis software. Body weight, height, and waist and hip circumferences were measured. Waist was measured at the umbilicus (wstumb), iliac crest (wstili), and mid-way between the iliac crest and lowest rib (wstwst). Waist-to-hip ratios (WHRUMB, WHRILI, and WHRWST) were also calculated. Women who were both homozygous for (CAG)<sub>n</sub> ≥ 25 and homozygous for the two most common (GGN)<sub>n</sub> (=17 or =18) had higher wstumb (93.43±14.86 vs 85.02±10.19, p=0.003), wstili (97.02±10.83 vs 91.61±10.36, p=0.014), wstwst (88.20±11.68 vs 80.33±8.64, p=0.000), WHRUMB (0.908±0.081 vs 0.869±0.060, p=0.012), WHRWST (0.859±0.071 vs 0.821±0.052, p=0.005), and hip (102.61±10.36 vs 97.77±7.93, p=0.014) compared to women without this allele combination. The odds ratio (OR) related to the upper 25% of women with this allele combination was significant for wstumb (OR=5.29, 95% CI 1.48-18.92), wstwst (OR=3.62, 95% CI 1.15-11.39), WHRUMB (OR=5.61, 95% CI 1.57-20.02), and WHRWST (OR=5.29, 95% CI 1.48-18.92). Men who had both (CAG)<sub>n</sub> ≥ 23 and rare (GGN)<sub>n</sub> (≠ 17), had a higher mean WHRUMB (0.990±0.042 vs 0.971±0.049, p=0.043) and WHRILI (0.991±0.052 vs 0.967±0.047, p=0.017) compared to men without this allele combination. The OR related to the upper 25% of men with this allele combination was significant for WHRILI (OR=6.05, 95% CI 1.19-30.82) and WHRWST (OR=6.05, 95% CI 1.19-30.82). AR (CAG)<sub>n</sub> and (GGN)<sub>n</sub> allele combinations are strongly associated with obesity indices in older adults. Sponsored by NRICGP/USDA Award No. 97-35-207-4619 and Utah Agricultural Experiment Station.

**A duplication at the D10S526 microsatellite locus generates a bimodal distribution of allele sizes.** *P. Bender*<sup>1</sup>, *W. Beggs*<sup>1</sup>, *L. Toji*<sup>1</sup>, *J. Beck*<sup>1</sup>, *D. Long*<sup>2</sup>, *F. de Waal*<sup>2</sup>, *R. Johnson*<sup>1</sup>. 1) Coriell Institute, Camden, NJ; 2) Living Links Center, Yerkes Regional Primate Research Center, Emory University, Atlanta, GA.

The Coriell Institute uses the D10S526 microsatellite tetra-nucleotide repeat in the routine genotyping of its cell lines for DNA identification because of its high heterozygosity. Initial genotyping of 200 alleles indicated a heterozygosity of .84 and a PIC value of .83. Now that thousands of alleles have been genotyped, it is obvious that there is a bimodal distribution of allele sizes. Alleles are either 190 to 218 bp (short) or 226 to 258 bp (long). No alleles have been observed at 222 bp. Individuals occur who are homozygous either for the long and short alleles or are heterozygous, with long and short alleles. The molecular basis for this bimodal distribution could be explained by an ancient duplication of a short allele, generating the longer alleles with two polymorphic repeat regions separated by a short non-repetitive sequence. To investigate this possibility, short and long alleles were isolated from polyacrylamide gels and sequenced. The sequence results demonstrate that whereas the short alleles have a single polymorphic tetra-nucleotide repeat, long alleles have two polymorphic repeat regions separated by an invariant dinucleotide. This finding complicates the interpretation of allele scoring and increases the number of long alleles. Two long alleles of the same size may, therefore, not be identical. For example, a 20 repeat allele could result from the presence of an 11 repeat in the first region followed by a 9 repeat in the second region, or a 9 repeat in the first region followed by an 11 repeat in the second region. Such homonym alleles have been identified by sequence analysis. The origin of this duplication was investigated by genotyping non-human primates. Twenty-four chimpanzees (*Pan troglodytes*) and one bonobo (*Pan paniscus*) were genotyped for the D10S526 microsatellite. None of the 50 alleles analyzed were long. Thus, either the long allele is not present in primates or occurs at a low frequency. These results indicate that the duplication may be a late event in human evolution and that the long alleles are specific to the *Homo sapiens* lineage.

**Evolution of Microsatellite Loci and Past Demography of Modern Humans.** *M. Kimmel*<sup>1</sup>, *A. Renwick*<sup>1</sup>, *R. Deka*<sup>2</sup>, *R. Chakraborty*<sup>3</sup>. 1) Dept Statistics, Rice Univ, Houston, TX; 2) Dept Environmental Health, Univ of Cincinnati, Cincinnati, OH; 3) Human Genetics Ctr, Univ Texas at Houston, Houston, TX.

The group involving these authors, previously analyzed several large data sets involving di-, tri- and tetranucleotide repeat loci throughout the human genome. We also developed new statistical tools to analyze these data. In the present communication, we carry out a comparative analysis of these data sets, in the context of the molecular evolution of short repeat-DNA and past demography of populations harboring these loci. The tools we use involve decomposition of genetic diversity, coalescent simulations under variable rate and pattern of mutation (e.g. admixture of large-step mutational changes, constraints etc.), as well as analysis of ascertainment bias of human versus primate microsatellites. The conclusions can be summarized as follows: (i) Di- and tetranucleotide loci fit the stepwise mutation models with mutation rate varying from locus to locus and large allele-size changes occurring in about one-tenth of mutants. (ii) Trinucleotide loci are better fitted by models involving constraints on the number of repeats. (iii) Mutation rates differ among these three types of loci. In anonymous or non-gene associated loci, the highest mutation rate occurs in dinucleotides, the lowest in tetranucleotides. However, in disease-related trinucleotides, even in normal subjects, the mutation rate seems to be highest than in anonymous loci. (iv) The data seem to be consistent with the hour-glass scenario of demographic expansion of modern humans. (v) The extent of difference observed between diversities of homologous human and primate microsatellite loci cannot be explained by ascertainment bias alone. It suggests a higher mutation rate of human, as compared to primate, microsatellites. The results are discussed in view of other findings concerning evolution of human and non-human microsatellites. (Research supported by NIH grants GM 41399, GM 45816 and GM 58545).



**Multiple haplotypes in DM2 indicate multiple founder mutations.** *C.L. Liquori<sup>1</sup>, K. Ricker<sup>2</sup>, J.F. Jacobsen<sup>1</sup>, L.J. Rasmussen<sup>1</sup>, K.A. Dick<sup>1</sup>, J.C. Dalton<sup>1</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) Department of Neurology, University of Würzburg, Germany.

Myotonic dystrophy (DM) is the most common form of muscular dystrophy in adults. The existence of more than one genetic type was recognized after genetic testing became available for myotonic dystrophy type 1 (DM1). DM1 is caused by a CTG expansion within the 3' UTR of the *myotonia dystrophica-protein kinase* gene. The mechanism by which this untranslated expansion causes DM remains unclear. To better define DM pathogenesis, we have studied a second genetic form of DM, myotonic dystrophy type 2 (DM2). We previously mapped the DM2 locus to chromosome 3q21 and refined the region to 2cM with 10 recombinant chromosomes. Linkage disequilibrium analysis in parent-offspring trios from 64 DM1-negative families narrowed the region to ~320 Kb with several markers in linkage disequilibrium (*CL3N58* ( $p < 0.000001$ ), *CL3N59* ( $p = 0.0001$ ), *CL3N84* ( $p = 0.0001$ ), and *CL3N99* ( $p = 0.0075$ )). We discovered that DM2 is caused by a CCTG expansion in intron 1 of the *zinc finger protein 9* gene (see Ranum, *et al.*). We determined haplotypes for 74 families with genetically-confirmed DM2 using 18 new STR markers in the DM2 region. All of the families are Caucasian with the majority of German descent. We found two distinct haplotypes. 60 families share haplotype A, which spans ~425 Kb. Of those families, a subset show an ancestral recombination event, reducing the size of the shared chromosomal region to ~320 Kb. 14 families share haplotype B, which spans ~800 Kb. Families with either haplotype have consistent clinical features and the CCTG expansion. Fine mapping with additional markers will be done to determine if the A and B haplotypes converge close to the mutation. We have also identified 23 families that lack both the DM1 and DM2 expansions, indicating the existence of at least one additional DM gene (DM3).

**Expression and population studies of the TATA-box binding protein polyglutamine region at normal and expanded lengths.** *S.J. Reid<sup>1</sup>, M.I. Rees<sup>1</sup>, W.M.C. van Roon-Mom<sup>2</sup>, A.L. Jones<sup>3</sup>, M.E. McDonald<sup>4</sup>, G.T. Sutherland<sup>1</sup>, M.J. Owen<sup>3</sup>, M. Dragunow<sup>5</sup>, R.G. Snell<sup>1</sup>.* 1) Department of Molecular Medicine, University of Auckland Medical School, Auckland, New Zealand; 2) Department of Anatomy and Radiology, University of Auckland Medical School, Auckland, New Zealand; 3) Department of Psychological Medicine and Medical Genetics, University of Wales College of Medicine, Cardiff, UK; 4) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, USA; 5) Department of Pharmacology, University of Auckland Medical School, Auckland, New Zealand.

Several inherited, late onset, neurodegenerative diseases are associated with expansion of CAG repeats within coding regions, resulting in pathogenic glutamine tracts in the respective proteins. TATA-box binding protein (TBP) contains a polymorphic polyglutamine (polyQ) coding repeat ranging in size from 25-42, clearly extending into the pathogenic (>37) threshold repeat length observed in polyQ disorders. Indeed, a de-novo polyQ expanded TBP allele was recently reported in a patient with a sporadic, early-onset neurodegenerative disorder, showing that a disease phenotype is associated with TBP polyQ expansions. To assess the population distribution of TBP alleles, we carried out a large population study of the TBP polyQ repeat coding sequence, which did not reveal expansions larger than 41, suggesting a biological selection against further expansion, or that the expansion has reached a stable equilibrium. However, allele frequency studies of different human populations indicate that the repeat is at least ancestrally unstable. To identify the critical repeat length in TBP that may result in a disease with long term population effects, a cellular model of protein misfolding was used, expressing full length TBP carrying polyQ expansions. We found that insoluble intracellular inclusions are formed in a repeat length dependant manner, with a threshold level of inclusion formation. These results show that expansions within TBP produce a protein with altered structural properties in a manner reminiscent of other polyQ human diseases.

**Association of the Apolipoprotein E (APOE) Gene with Age-Related Macular Degeneration (AMD): A pooled case-control study.** *S.A. Schmidt<sup>1</sup>, M.B. Gorin<sup>2</sup>, C.C.W. Klaver<sup>3</sup>, K.W. Small<sup>4</sup>, J.L. Haines<sup>5</sup>, E.A. Postel<sup>1</sup>, A.M. Saunders<sup>1</sup>, C.M. van Duijn<sup>3</sup>, D.E. Weeks<sup>2</sup>, R. Ferrell<sup>2</sup>, A. Agarwal<sup>5</sup>, M.A. Pericak-Vance<sup>1</sup> for the APOE-AMD Consortium.* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Univ of Pittsburgh Graduate School of Public Health/School of Medicine, Pittsburgh, PA; 3) Erasmus Univ Medical School, Rotterdam, The Netherlands; 4) The Jules Stein Eye Institute, Univ of California, Los Angeles, CA; 5) Vanderbilt Univ Medical Ctr, Nashville, TN.

AMD is a complex genetic disorder affecting older adults. The APOE4 allele has been reported to have a protective effect on AMD risk, while the APOE2 allele may increase disease risk. We have combined three independent data sets of Caucasian patients and controls (two US and one European) to study the role of APOE in AMD and to examine whether familial vs. sporadic cases of AMD represent different disease etiologies based on APOE-associated risks. Analysis of an additional US AMD data set is ongoing in order to strengthen and refine our results. Based on the currently available data comparing n=524 AMD cases and n=1187 controls with respect to APOE genotypes, we were able to confirm the protective effect of the APOE4 allele (age- and sex-adjusted odds ratio (OR) 0.54, 95% confidence interval (CI) 0.41-0.72, p=0.0001). The effect of APOE4 did not differ significantly between males and females and was consistent across all age groups. This overall effect was primarily due to cases (n=342) with a familial etiology (OR 0.43, 95% CI 0.30-0.60, p=0.0001). When only sporadic cases (n=182) were examined, the APOE4 effect was no longer statistically significant (OR 0.81, 95% CI 0.53-1.23, p=0.32). Evidence for an increased risk conferred by the APOE2 allele was found only for sporadic male patients (OR for male patients 2.72, 95% CI 1.38-5.35, p(interaction)=0.02 from logistic regression). In conclusion, these data strongly confirm that the APOE4 allele reduces the risk of AMD, particularly for patients with a positive family history of the disease. For male patients without a family history, the APOE2 allele appears to increase the disease risk, whereas this effect is absent in females.

**Utility of Hereditary Hemochromatosis (HH) Mutation Detection Tests in patients and relatives from a region with low prevalence of disease causing mutations.** *F.F. Fay<sup>1</sup>, M. Campodonico<sup>1</sup>, J. Daruich<sup>2</sup>, H. Tanno<sup>3</sup>, J. Rey<sup>2</sup>, J. Sorda<sup>2</sup>, O. Fay<sup>1</sup>, J. Findor<sup>2</sup>.* 1) Dept Molecular Biol, CIBIC, Rosario, Argentina; 2) Hospital de Clinicas San Martin - Buenos Aires; 3) Hospital del Centenario - Rosario.

**Introduction:** HFE gene mutation detection is used in populations from Northern-European origin as a confirmatory test for HH, and in the pre-symptomatic diagnosis of their first-degree relatives. Its utility has not been established in settings with lower prevalences of disease causing mutations.

**Aims:** To determine the presence of HFE gene mutations (C282Y and H63D) in patients with HH and their relatives, and to establish the clinical utility of this test in our region.

**Material and Methods:** C282Y and H63D mutations were investigated in 35 HH patients. From 15 patients (index cases) 30 first degree relatives were also studied (26 sons from 12 patients and 4 brothers from the other three). Mutation detection was performed using a multiplex PCR.

**Results:** From the 35 studied patients, 12 (34%) were C282Y homozygous, 7 (29%) were H63D heterozygous, 1 (3%) was double-heterozygote, 4 were C282Y heterozygous, 3 H63D heterozygous and 8 were wild type. Regarding the studied relatives, there were 8 with HH related mutations; 2 Hom282, 2 Hom63, and 4 Double-Het. The remaining 22 relatives had no disease causing genotypes.

**Discussion:** In HH patients the sum of Hom C282Y, Hom H63D and Double-Heterozygotes accounts for the 57% of the cases, which could justify the use of this tool as a diagnostic complement. The greater benefit is seen in the screening of relatives from those patients with one of the disease causing genotypes, since in 60% (6/11) of the latter there were relatives with genetic predisposition. On the other hand, in 38% (8/21) of the studied relatives we found disease causing mutations. These persons are being evaluated periodically to detect any increase in iron deposits, so preventive measures could be taken in advance.

Program Nr: 1423

**Informed choice and prenatal screening: comparison of same day vs return visit testing.** *T.M. Marteau, E.R. Dormandy, S. Michie.* Psychology & Genetics Res Grp, King's College London, London, England.

*Objective:* To test the hypothesis that rates of informed choice are higher when testing requires a return visit than when conducted as part of a routine antenatal appointment.

*Design:* Prospective, descriptive study.

*Setting:* Two hospitals in England.

*Participants:* 1500 women offered serum screening for Down syndrome.

*Outcome measures:* Informed choice, derived from measures of **knowledge** about the screening test, **attitudes** towards undergoing the test, and test **uptake**.

*Results:* Rates of informed choice were higher for same day than for return visit testing (64% vs 44%, CI<sub>95</sub> of difference 16, 26%). Regardless of levels of knowledge, same day testing did not result in more women with negative attitudes having the test than did return visit testing (9% vs 8%). However, return visit testing resulted in more women with a positive attitude towards testing **not** having the test than did same day testing (21% vs 7%, CI<sub>95</sub> of difference 9, 16%).

*Conclusion:* Screening offered as part of a routine visit appears to facilitate informed choice amongst those with positive attitudes towards screening without impairing informed choice amongst those with negative attitudes.

**Carrier screening for four common diseases in the South African Ashkenazi Jewish population.** *R. Kerr, C. Graf, F. Essop, B. Dangerfield, A. Krause.* Department of Human Genetics, School of Pathology, South African Institute for Medical Research and the University of the Witwatersrand, Johannesburg, South Africa.

Several inherited diseases have been recognized as occurring more commonly in the Ashkenazi Jewish population, including Tay-Sachs disease (TSD), cystic fibrosis (CF), Fanconi anaemia group C (FA-C) and Canavan disease (CD). Population carrier frequencies for the four diseases have been determined in other countries. Worldwide data reflect the following carrier frequencies: CF (1/24), TSD (1/27), CD (1/58) and FA-C (1/66). Ashkenazi Jews in South Africa may have some genetic differences from other Ashkenazi groups - e.g. Bloom syndrome, which has a carrier frequency of 1/80 in USA Ashkenazi Jews, has never been reported in South Africa. In order to offer carrier screening and accurate risk calculations for these conditions as part of our routine diagnostic service, it is essential that we generate carrier frequencies specific to the population we serve. Approximately 250 Ashkenazi Jewish South African individuals are being screened for the population-specific mutations which have been identified for each disease. Two CF mutations (DF508 and W1282X), two TSD mutations (intron 12 G→C splice mutation and exon 11 4bp insertion mutation), two CD mutations (C693A and A854C) and one FA-C mutation (IVS4+4A→T) will be tested for. Screening for Canavan disease in the South African Ashkenazi Jewish population reveals a carrier frequency of 1/93, which is approximately 2 times lower than frequencies reported elsewhere. TSD carrier frequencies for the South African Ashkenazi Jewish population have been determined using the enzyme assay (1/26). This rate will be refined using molecular techniques.

**Iranian Human Mutation Gene Bank.** *M.K. Javan, M. Neishabouri, A. Hajibeigi, K. Kahrizi, Y. Shafaghati, F. Pourfarzad, N. Nikzad, H. Najmabadi.* Genetics, Univ. of welfare and Rehabilit, Tehran, Tehran, Iran.

For over a decade prevention of the common genetic disorders was a national programme in Iran. The result of our ten years study on carrier identification and prenatal diagnosis of genetic diseases, has revealed the existence of a wide spectrum of mutations for certain diseases in this country. This is a reflection of Iran's long history of foreign invasion, immigration, cultural exchange and also the high prevalence of interfamilial marriages, creating a multiethnic society with the highly heterogeneous gene pool. During the last four years investigation we have established a DNA bank of all genetic disease with Mendelian mode of inheritance studied in Iran. Some of the samples are assigned to common or novel mutations and some others belong to patients with clinical profiles associated with a particular genetic disease but unidentified mutation. This bank stores patient and his/her first degree relatives DNA together with a comprehensive pedigree and clinical profile for each sample. To improve our diagnostics, we found it essential to establish a link between our findings and the other research projects elsewhere in the world by presenting our experimental projects and this DNA bank on line. Our web site as well as providing opportunities for us to collaborate with outside, offers a free of charge valuable sample resource to all the researchers in the world, who are working on various aspect on genetic disorders from prenatal diagnosis to gene structure and function. This article introduces the structure and the content of this DNA bank.

**An effective pooling strategy for feasible newborn screening of *GBJ-2* gene mutations.** *M. Caggana*<sup>1</sup>, *S. Duva*<sup>1</sup>, *H. Ostrer*<sup>2</sup>, *C. Oddoux*<sup>2</sup>, *R. Ruben*<sup>3</sup>, *K. Pass*<sup>1</sup>. 1) Wadsworth Ctr, NYSDOH, Albany, NY; 2) New York University School of Medicine, NY, NY; 3) Albert Einstein College of Medicine, Bronx, NY.

Congenital deafness affects approximately 1 per 1,000 newborns in the US. The causes of non-syndromic deafness are heterogeneous, with genetic factors accounting for 50-75%. In the absence of screening, the average age at diagnosis is 14 months, thus by waiting for a conventional diagnosis, an important window of opportunity for early intervention is missed. Approximately one-half of autosomal recessive congenital deafness is caused by mutations in the *GBJ-2* gene, which codes for the gap junction protein, Connexin-26. Mandatory hearing screening has been implemented recently in several states including New York. We undertook a pilot project to assess the feasibility of screening all newborns for two *GBJ-2* mutations (35delG, a pan-ethnic mutation and 167delT, a mutation more frequently found in individuals of Ashkenazi Jewish descent). A consecutive series of anonymous newborn dried blood specimens were tested (N=2,071) for both mutations by allele-specific oligonucleotide hybridization in single wells. This sample is representative of a 2-day workload for the NYS newborn screening program. Once the positive samples were identified, they were blinded and pooled by a factor of 8 thereby reducing the number of amplification procedures in 96-well plates from 22 to 3. All positive samples in the individual experiment were correctly identified in the pooled experiment, suggesting that it is plausible to sufficiently automate and manage this workload. The carrier frequencies of 35delG for Whites (n=1,111), Blacks (n=401), and "others" (n=107) were 2.16%, 0.25%, and 1.87% respectively. One homozygote for 35delG was identified. The overall allele frequency for del35G in this screening sample is 0.7%. For 167delT, the carrier frequencies were 0.36% in Whites, 0.28% in Hispanics, and 0.93% in "others". No homozygotes for 167delT were identified. The overall allele frequency for 167delT in this screening sample was 0.14%. Use of DNA analysis with audiometry will serve to "rule-in" the diagnosis of deafness permitting parents to pro-actively enroll their infants in intervention programs.



**Perilymphatic fistulae associated with *DFNBI*-related hearing loss.** C. Oddoux<sup>1</sup>, R.J. Ruben<sup>2</sup>, H. Ostrer<sup>1</sup>. 1) Division of Human Genetics, New York University School of Medicine, New York, NY; 2) Department of Otolaryngology, Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY.

Mutations in the *DFNBI* gene produce prelingual hearing loss that is always sensorineural and is typically moderate to severe. Vestibular function is unaffected and no other phenotypic abnormalities have previously been observed. Little is known about the course of the hearing loss as most of the published studies have focused on children or adults evaluated well after the onset of deafness and these patients were not followed prospectively. Four children carrying two mutant alleles (combinations of 30delG and/or 167delT) were detected by *DFNBI*-mutation analysis of 26 children with sensorineural hearing loss who were followed prospectively by a single otolaryngologist. Retrospective review of their records revealed a common anatomic defect in the footplate of the oval window of five middle ears—a perilymphatic fistula. This defect was associated with progressive hearing loss in three of the four children. The fourth child had a particularly severe course of otitis media which could have accelerated progression of hearing loss. In some cases closure of the leak was associated with an increase in speech discrimination.

These observations suggest that individuals carrying two mutant *DFNBI* alleles may not be identified by newborn screening programs that use auditory brain stem responses to test for hearing loss. As a result, children with *DFNBI*-related deafness might be missed during the first year or two of life because the assumption may be made that their hearing is normal. This in turn would have a negative impact on the child's language acquisition. Early identification may require a direct genetic test for *DFNBI* mutations in the newborn period. The favorable responses of patients to the surgical interventions presented here, suggests that this issue merits further study to determine whether early intervention can delay the onset of hearing loss possibly to postlingual period which would have a major impact on language acquisition.

**Pendrin gene mutation analysis for children with sensorineural hearing loss.** *V. Lip<sup>1</sup>, N. Lindeman<sup>1</sup>, M.A. Kenna<sup>2</sup>, B-L. Wu<sup>1</sup>.* 1) Department of Laboratory Medicine & Pathology, Children's Hospital, Boston, MA; 2) Department of Otolaryngology, Children's Hospital, Boston, MA.

**BACKGROUND:** Pendred syndrome (PDS) is an autosomal recessive disorder characterized by congenital sensorineural hearing loss, cochlear malformation, and thyroid goiter, and is due to mutation of the Pendrin gene at 7q31. Currently, diagnosis is predicated upon clinical and radiological findings. Because the thyroid disease does not develop until late childhood/early adulthood and can progress to cancer, early molecular diagnosis may lead to aggressive thyroid monitoring and preventive therapy. Molecular diagnosis is facilitated by the clustering of most (~60%) significant mutations (L236P, K369E, A372V, E384G, T416P, 2111insGCTGG, T721M, and H723R) within exons 6, 9, 10, and 19. **METHODS:** We developed a two-step assay for Pendrin mutations, using PCR with primers flanking/overlapping the "hot spot" exons 6, 9, 10, and 19, followed by automated sequence analysis. Blood samples from 63 patients with hearing loss were tested. **RESULTS:** Pendrin exons 6, 9, 10, and 19 were successfully amplified from all 63 samples. Mutations were detected in 5 samples. These included a known mutation (T416P), a silent mutation (L729L), an intronic mutation, and a novel mutation (F335L), found in two unrelated patients. **CONCLUSION:** Molecular diagnosis of Pendred syndrome by PCR-sequencing is feasible and diagnostically useful. In addition to detecting patients with known mutations, we have identified a novel mutation (F335L) in two unrelated deaf children. Longer clinical follow-up will be needed to determine if this mutation is associated with Pendred syndrome.

Program Nr: 1429 from the 2001 ASHG Annual Meeting

**The European Directory of DNA Laboratories (EDDNAL).** *C.S. Albott, L. Van Maldergem.* Centre de Gntique Humaine, Institut de Pathologie et Gntique, Loverval, BELGIUM.

A rapid increase in the number of genetic diseases open to a DNA based diagnostic test has given rise to the need for a comprehensive directory of European laboratories and a listing of the services they can offer. In 1995, the European Directory of DNA Laboratories (EDDNAL) was established to meet this need. Our work investigates the diversity among European diagnostic centers and the way in which the EDDNAL web-site ([www.eddnal.com](http://www.eddnal.com)) is able to provide information on diagnostic services provided in the different EU member states. We performed a review of the literature to ascertain the differences among European diagnostic laboratories of Europe as well as the problems specific to these countries. We describe how the EDDNAL registry provides information on the Access, Availability and Quality Ascertainment throughout the European community. In addition, we present schemes for circumventing the previously ascertained problems. The on-line directory is designed to promote information exchange between European centres and to improve awareness of the availability of services for rare genetic conditions. The development of the EDDNAL database and web-site should result in an overall decrease in the total cost of DNA diagnoses of rare genetic disorders due to a European concerted approach based on the prevalence of such disorders.

**A web-based expert system for gene mutation nomenclature.** *J. Li, W. Kimberling and Center for the Study and Treatment of Usher Syndrome.* Dept. of Genetics, Boystown National Research Hospital, Omaha, NE.

Unequivocal gene mutation nomenclature is essential for reporting and testing sequence changes (mutations and polymorphisms) efficiently and accurately. It is also critical for creating and maintaining integrated locus-specific mutation databases. A nomenclature system has been recommended and currently largely accepted to describe sequence changes at DNA, RNA and protein levels. It has stimulated the consistent and explicit description of sequence changes. However, reports with not quite precise nomenclature of sequence changes can still be found partially due to authors failing to abide by the nomenclature rules strictly. Thus therefore retards the development of integrated mutation databases. To assist scientists to follow the rules of nomenclature stringently and easily, we are developing a web-based expert system for gene mutation nomenclature. Through an interactive question and answer process, one can easily, accurately, and consistently obtain the proper names of sequence variations. The expert system is currently being evaluated as a method of providing consistent nomenclature for creating and maintaining Usher syndrome mutation databases, but would be applicable to all genetic disorders. The system is programmed with Active Server Pages (a web programming language) interacting with both the locus information database and the oligo primer database for Usher syndrome that developed in Microsoft SQL server. It is currently accessible on the Boys Town National Research Hospital intranet and will be accessible on the World Wide Web in the future. In addition, the issues about the numbering of nucleotides and how to develop a consistent numbering system of nucleotides among different reference sequences in a long term will be also discussed.

**The COPPER plate system: Effective use of DHPLC for clinical application.** *H. Yoshihashi, T. Suzuki, K. Kosaki.*  
Dept of Pediatrics, Keio Univ Sch Med, Tokyo, Japan.

The more the molecular geneticists unravel the genetic basis of inherited diseases, the more the practicing clinical geneticist get frustrated because they are confronted with a situation in which molecular diagnostic tests cannot be offered to the patients simply because of the cost and labor associated with such testing. A cost-effective and automated strategy needs to be implemented. Denaturing high-performance liquid chromatography (DHPLC), the new technology, based on temperature-modulated liquid chromatography and a high-resolution matrix was implemented in a way suitable for clinical application. Primers were designed so that all of the primers would have the same annealing temperature. In this way, all the exons can be amplified simultaneously in a single PCR machine. Primer pairs were aliquoted on a 96-well format PCR plate referred to as COPPER plate (Condition-Oriented-PCR primer-Embedded-Reactor). The COPPER plate was accompanied with a corresponding computer programs optimized for DHPLC analysis of the particular PCR product. COPPER plates and accompanying computer programs were developed for analysis of the NEMO gene (incontinentia pigmenti), the JAGGED1 gene (Alagille syndrome), and the P63 gene. All the exons could be analyzed sequentially by the optimized method and all the operation were completely automatic once the operator sets the plate and starts the "gene-specific" analysis program. The P63, NEMO, and JAGGED1 package consists of 8, 9, and 30 primer pairs. Using the COPPER plate, we have identified several mutations: C835T (R279C) mutation in P63, C550T (R184C) mutation in JAGGED1, and C184T (R62X), 1116delT, and 1161-1167insC mutations in NEMO. COPPER plates for the DHPLC technology, when complemented with the COPPER plate system, offers an ideal tool for mutation analysis in clinical applications. The key aspects to setting the operating parameters will be discussed.

**Use of conformation sensitive gel electrophoresis (CSGE) for mutation analysis of complex connective tissue**

**genes: COL1A1, COL1A2, COL2A1, COL11A1 and FBN1.** *J. Korkko<sup>1</sup>, I. Kaitila<sup>2</sup>, D. Prockop<sup>1</sup>, L. Ala-Kokko<sup>1</sup>.* 1) Ctr Gene Therapy, Tulane Univ Health Sci Ctr, New Orleans, LA; 2) Dept Clin Genet, Helsinki Univ Hosp, Finland.

Fibrillar collagens provide structural support for connective tissues. Collagen I is a major protein of skin, tendon and bone and collagen II is the main component of cartilage and the vitreous of the eye. Collagen XI found in same tissues as collagen II, but it is a minor component. The genes coding for these proteins are complex in that they consist of over 50 exons and have a highly repetitive structure. Therefore, it has been difficult to establish a practical and reliable method for mutation detection in these genes. During the last few years we have adapted the procedure of conformation sensitive gel electrophoresis (CSGE) for these genes and analyzed patients with different osteochondrodysplasias for mutations. The mutation detection rates are the following: COL1A1/COL1A2 analysis in patients with osteogenesis imperfecta (OI): OI type I 88%, OI type II 83%, OI type III 83%, OI type IV 73% and OI vs abuse 4%. Conclusion: The vast majority of the OI is caused by collagen I gene defects. In OI vs child abuse only 2 of 52 patients had COL1A1/COL1A2 defect. COL2A1 mutations were found in 95% of the patients with hypo- and achondrogenesis II, in 64% of the patients with spondyloepiphyseal dysplasia and in 75% of patients with Kniest dysplasia. Conclusion: Achondrogenesis II/hypochondrogenesis is virtually always caused by COL2A1 mutations. Collagen II defects are common, but not the only cause of SED/SEMD. 74% of the patients with Stickler or Marshall syndromes have mutations in either the COL2A1 or COL11A1 gene. In general Stickler syndrome seems to be more commonly associated with the COL2A1 gene, and Marshall syndrome with the COL11A1 gene. We have initiated testing of CSGE to detect mutations in the FBN1 gene in patients that meet the revised diagnostic criteria for Marfan syndrome (DePaepe et al., Am J Med Genet, 1996). Preliminary results indicate that CSGE is highly sensitive (about 90%) and specific in detection of mutations in the FBN1 gene.

**The use of unequal expression as a rapid diagnostic tool for neurofibromatosis 2.** *H.-O. Nguyen, B. Heinrich, M. MacCollin.* Department of Neurology, Massachusetts General Hospital, Charlestown, MA.

Neurofibromatosis 2 (NF2) is an autosomal dominant disorder characterized by the development of multiple nervous system tumors. Although pre-symptomatic diagnosis of NF2 patients affords better final outcomes, current molecular diagnosis methods based on exon scanning are costly, time-consuming and frequently fail to detect underlying mutations. We have previously shown that 83% of known mutations are underexpressed in messenger RNA from patients with NF2. We thus explored the use of altered expression of disease bearing alleles as a rapid and simple molecular test for NF2. Three assays in the untranslated region (UTR) of the *NF2* gene were developed utilizing polymorphisms which create or destroy restriction enzyme recognition sites. Heterozygosity in genomic DNA amongst a cohort of 31 unrelated individuals ranged from 10 to 25%, with 14 individuals polymorphic at least in one assay. Total cellular RNA from peripheral blood leukocytes was reverse transcribed using oligo-dT primers and amplification of alleles in mRNA was compared to that in genomic DNA. Qualitative analysis on ethidium bromide stained gels was frequently adequate to detect unequal expression in heterozygous NF2 patient samples compared to unaffected controls. Quantitative levels of expression were determined using FAM label of a single primer. Differences in expression levels were evaluated by an automatic sequencer using area-under-curve values associated with each allele. Although unequal expression was apparent in heterozygous NF2 patient samples, mixing experiments using known homozygous samples revealed that this method consistently underestimated the over represented allele. Delay in extraction of RNA from whole blood samples augmented unequal expression by decreasing the amount of the under represented allele. Unequal expression of disease bearing alleles can be easily assayed in peripheral blood specimens, and may be a powerful method of rapid and inexpensive molecular diagnosis in this and other autosomal dominant disorders.

**SMA carrier detection using TaqMan technology : Implications for genetic counseling.** *E. Vial, P. Burllet, L. Viollet, A. Munnich, S. Lefebvre.* INSERM U393, Hopital Necker Enfants Malades, Paris, France.

Spinal muscular atrophy (SMA) is an inherited neuromuscular disease characterized by specific degeneration of the anterior horn cells of the spinal cord leading to muscle atrophy. It is the second most common fatal autosomal recessive disorder, with a carrier frequency of 1/40. In most cases (>95%), SMA is caused by the homozygous loss of the telomeric survival motor neuron gene (SMN1) located within a duplicated element in a highly unstable region of chromosome 5q13. All patients and 95% of control individuals carry the copy gene, SMN2. We report a single step duplex real-time quantitative PCR with the Taqman technology for the detection of SMN1 heterozygous status. SMN1 gene dosage is based on a single base pair difference between SMN1 and SMN2 exon 7. This Taqman method relies on the comparison of the amount of the very specific PCR product generated from the SMN1 exon 7 with the amount of product generated from the albumin (ALB) disomic reference gene at exponential phase. A theoretical single cycle difference between these two amounts shows an heterozygous deletion of SMN1 exon 7. Studying a series of 108 parents (obligate carriers) we found that 5,5% (6/108) of them had two SMN1 genes, indicating either a cis duplication, a neomutation (de novo deletion) or a germinal mosaicism. This rapid test is currently used in our laboratory for genetic counseling in SMA families and for SMN1 gene dosage in SMA patients prior to mutation screening. The test has already been offered to 20 couples. One of the non-related individuals with a theoretical risk of 1/40 was found to carry an heterozygous deletion of SMN1 exon 7. Her husband ( the SMA relative) had two SMN1 copies with a starting theoretical risk of 1/2. The probability for this couple to give birth to an affected child raises from 1/320 to 1/80. The implications of these data for genetic counseling is that one must take into consideration both parents in the evaluation of the risk and in the decision to propose prenatal diagnosis.



**Contacting Huntington carriers and non-carriers for research purposes after pre-symptomatic testing.** C.C. Verschuuren<sup>1</sup>, E.H. Sikkens<sup>1</sup>, J.C.H. Oostrom<sup>2</sup>, K.L. Leenders<sup>2</sup>, A.J. vanEssen<sup>1</sup>. 1) Clinical Genetics department, University Hospital Groningen, the Netherlands; 2) Neurology department, University Hospital Groningen, The Netherlands.

*Introduction:* Pre-symptomatic testing for Huntington disease is done in our hospital at the department of Clinical Genetics according to the international protocol. After the testing procedure is completed there is usually no further contact with the tested persons. For a research project at the Neurology department and P.E.T.Center pre-symptomatic Huntington carriers and controls were needed. This confronted us with an ethical dilemma because an unannounced contact can give emotional distress for a carrier and a non-carrier. To legitimize the contacting of this group for research purposes we scored their reactions to this unannounced request for participation to a neuro-imaging investigation.

*Methods:* After approval of the ethical commission 70 persons (31 carriers, 39 non-carriers) counseled between 1994 and 2000 were contacted by mail. This mailing contained an introduction letter of the clinical geneticist, an information leaflet about the research project and a questionnaire. In this questionnaire the reaction to our unannounced mailing could be scored from very disturbing to no problem on a scale from 1 to 5. One follow-up call was made 3 weeks later to the carriers that had not responded. *Results:* The response rate was 75%(23/31) in carriers and 60%(23/39) in non-carriers. Telephone contacting of the carriers that had not-responded showed that 10%(3/31) were lost to follow up and 10%(3/31) had not responded because they had symptoms, which was an exclusion criterion for this study. 39%(9/23) of the responding carriers and 9%(2/23) of the responding non-carriers refused participation in the research project. In the questionnaire only 6.5% (3/46) of the responders scored (very) disturbing (score 1 or 2). All of these were carriers. *Conclusion:* Unannounced research request was fairly well accepted by (pre-symptomatic) carriers and non-carriers of Huntington disease with whom contact was closed because their counseling procedure at our department of clinical genetics was finished.

Program Nr: 1436 from the 2001 ASHG Annual Meeting

**Molecular testing for late onset neurodegenerative disorders: a health care professionals' perspective.** *RSR. Paiva, I. Lopes-Cendes.* Department of Medical Genetics, University of Campinas, Campinas, SP, BRAZIL.

**Objective:** To evaluate and compared opinions of five health professional groups about molecular testing for Huntington disease and spinocerebellar ataxias.

**Methods:** A structured questioner was sent to 530 health professionals, divided into 5 groups: 80 clinical geneticists, 100 neurologists, 100 psychiatrists, 150 psychologists and 100 nurses. All 530 professionals were practicing in clinics and/or hospitals in Sao Paulo State, Brazil.

**Results:** A total of 125 questionnaires (23.5%) were returned, varying from 30% of clinical geneticists to 18% of neurologists. Sixty-two % of the neurologists interviewed were currently following patients with one of these disorders; as opposed only 29% of geneticists and 16% of psychiatrists ( $p < 0.01$ ). Most geneticist and neurologist were aware that molecular tests are available for these disorders (96% and 84%, respectively). However, only 42% of the geneticists and 6% of the neurologists would feel comfortable dealing with issues related to molecular testing in their practice. Instead, the majority of professionals interviewed would refer their patients and family members to a specialized clinic for testing and counseling.

**Conclusion:** Although issues related to molecular testing for Huntington disease and spinocerebellar ataxia have been extensively discussed and recommendations have been issued by many medical societies, most health care professionals in Brazil, including clinical geneticists, do not feel comfortable dealing with testing and counseling issues in their practices.

**Clinical characteristics of individuals with germline mutations in *BRCA1* and *BRCA2*: Analysis of 10,000 individuals.** A.M. Deffenbaugh<sup>1</sup>, J.E. Reid<sup>2</sup>, M. Hulick<sup>1</sup>, B.E. Ward<sup>1</sup>, B. Lingenfelter<sup>1</sup>, K.L. Gumper<sup>1</sup>, T. Scholl<sup>1</sup>, S.V. Tavtigian<sup>2</sup>, D.R. Pruss<sup>2</sup>, G.C. Critchfield<sup>1</sup>, T.S. Frank<sup>1</sup>. 1) Myriad Genetic Laboratories, Salt Lake City, UT; 2) Myriad Genetics, Salt Lake City, UT.

In order to assess the characteristics of individuals in whom mutations in *BRCA1* and *BRCA2* were diagnosed in a clinical setting, we analyzed the results of 10,000 consecutive gene sequence analyses for mutations anywhere in the *BRCA1* and *BRCA2* genes (7491 analyses), or for three specific Ashkenazi Jewish founder mutations (2539 analyses), through information provided via a test requisition form. The presence and prevalence of mutations were correlated with personal and family history of cancer, ancestry, gender and invasive vs. noninvasive breast neoplasia. Mutations were identified in 1720 (17.2%) of the 10,000 individuals tested, including 968/4843 (20%) women with breast cancer and 281/824 (34%) with ovarian cancer. Mutations were as prevalent in high-risk women of African (25/133, 19%) and other ancestries as those of European ancestry (712/4379, 16%). *BRCA1* and *BRCA2* mutations were significantly less prevalent in women diagnosed with ductal carcinoma-in-situ ("DCIS") before age 50 than in women with invasive breast cancer diagnosed at a comparable age and with a comparable family history (13% vs. 24%,  $p = 0.0007$ ). Of the 74 mutations identified in individuals of Ashkenazi ancestry through full sequence analysis of both *BRCA1* and *BRCA2*, 16 (21.6%) were non-founder mutations including 7 in *BRCA1* and 9 in *BRCA2*. 21 of 76 (28%) men with breast cancer carried mutations of which more than one third occurred in *BRCA1*. We conclude that specific features of an individual's personal and family history can be used in a clinical setting to identify those most likely to carry mutations in *BRCA1* and *BRCA2*.

**The Carrier Clinic - a new model for oncological practice?** *A.T. ARDERN-JONES<sup>1</sup>, G. MITCHELL<sup>2</sup>, R.A. EELES<sup>1,2</sup>.*  
1) THE ROYAL MARSDEN NHS TRUST, LONDON, UK; 2) THE INSTITUTE OF CANCER RESEARCH  
SUTTON AND LONDON UK.

Patients with germ line mutations in cancer predisposition genes are at markedly increased risk of developing cancer. Furthermore, having developed cancer once does not preclude the development of further primaries. The early symptoms of cancer may be subtle or masquerade as benign disease. It is essential for nurses caring for families with gene mutations to have an understanding of cancer and its presentation in addition to the psychological support that is needed to realise the significance of symptoms that may seem non-threatening to the patient. This paper describes two clinical cases of BRCA gene carriers and one of a teenager whose mother is a carrier of the deleterious TP53 mutation where urgent referral was expedited following incidental reported symptoms by the patient during a follow up call from the clinical nurse specialist. Currently with the ever increasing information that pertains to gene carriers and their risks of developing other cancers it is essential that carriers have contact with a suitably informed person. At the Royal Marsden Hospital in London UK an annual follow up gene carrier clinic has been set up and this clinic acts as a gate keeping clinic for such families. This is staffed by a multidisciplinary team and both the nursing and medical staff have in-depth oncological as well as genetic training. Patients are kept in touch on a regular follow up basis by the nurse specialist and may contact the department at any time for any worries or concerns. The clinic team liaises closely with other practice specialists tailoring these contacts to the patients needs. We propose that families that have been found to carry cancer-predisposition genes are followed up in Carrier Clinics to provide up to date information and recommendations for management of these high-risk individuals.

**Clinical Genetic Testing for Mutations in the RB1 Gene using Direct Sequencing and Denaturing High Performance Liquid Chromatography.** *T. Ganguly<sup>1</sup>, C. George<sup>1</sup>, T. Dunbar<sup>1</sup>, L. Godmilow<sup>1</sup>, A. Ganguly<sup>1</sup>, K.*

*Nichols<sup>2</sup>.* 1) Department of Genetics, University of Pennsylvania, School of Medicine, Philadelphia, PA; 2) Children's Hospital of Philadelphia Pediatric Oncology, Philadelphia, PA.

Bilateral retinoblastoma, an embryonic retinal neoplasm presenting during early childhood, is associated with germline mutations in the RB1 tumor suppressor gene. Unilateral retinoblastoma, however, is due to somatic alterations, with only 10% of children harboring germline RB1 gene defects. To facilitate genetics counseling, we developed a highly specific and sensitive protocol for RB1 gene testing using DNA-based sequence analysis. In our assay, the RB1 coding sequences consisting of 27 exons, and all exon intron boundaries, are PCR amplified and sequenced directly. Using a set of 10 normal control DNA samples, we identified polymorphisms in introns 1, 3 and 17, the most commonly identified variants present in the RB1 Mutation database. Validation for mutation detection was performed using DNA derived from 20 retinoblastoma specimens for which underlying RB1 mutations had previously been identified. Following blinded analysis, we detected all mutations using direct sequence analysis. Next we performed a pilot study evaluating retinoblastoma patients with bilateral (n=5) or unilateral (n=5) disease. In 4 out of 5 (80%) patients with bilateral retinoblastoma, we identified germline RB1 mutations. For the unilateral cases, we identified 3 (60%) RB1 mutations, one of which was germline. These results are consistent with prior efforts reporting a 50-75% mutation detection rate for bilateral retinoblastoma patients. To improve the sensitivity and speed of mutation detection, and to reduce costs, we are currently evaluating the DHPLC system (Transgenomics) which is based on heteroduplex analysis of DNA fragments. Although analysis by DHPLC has been sensitive for most of the exons tested, the sensitivity is influenced by various parameters including DNA sequence, melting profile, run temperature and PCR reagents. Optimization of RB1 gene testing will facilitate genetics counseling and future management of retinoblastoma patients.

**DHPLC versus gel based SSCP/Heteroduplex: a comparative analysis for high throughput screening of HNPCC genes.** *J.F. Harvey<sup>1</sup>, C. Curtis<sup>1</sup>, P. Duncan<sup>1</sup>, D. Bunyan<sup>1</sup>, D. Eccles<sup>2</sup>.* 1) Wessex Reg Genetics Lab, Salisbury District Hosp, Salisbury Wiltsh, England; 2) Wessex Clinical Genetics Service, Princess Anne Hospital Southampton England.

We have previously described a rapid highly sensitive screen of 2 of the 6 mismatch repair genes mutated in HNPCC, hMLH1 and hMSH2. We transferred the 96 well microtitre plate format originally designed for high throughput gel based SSCP/heteroduplex analysis, to the dHPLC WAVE platform and detected all 24 unique mutations/polymorphisms previously found by gel based SSCP/heteroduplex analysis. We now describe a retrospective dHPLC study of 23 HNPCC families previously screened negative by SSCP/heteroduplex analysis. Mutations were detected in 7 unrelated patients (30 percent) including 3 stop and 4 splice site mutations. On re-running these mutated fragments by gel based SSCP/heteroduplex analysis we found 3 of the 5 mutations could now be detected, but only by using a range of gel conditions. Two mutations found in 4 unrelated patients remained undetected. Using a range of gel parameters is impractical and expensive for high throughput applications and highlights the variable sensitivity of gel based SSCP/heteroduplex analysis, questioning the continued use of SSCP/heteroduplex analysis in diagnostics for large scale mutation analysis.

**Results of a Stool BAT-26 Assay in People With HNPCC.** *S.J. Laken<sup>1</sup>, H.T. Lynch<sup>2</sup>, J.C. Urbanowski<sup>1</sup>, C. Deters<sup>2</sup>, A.P. Shuber<sup>1</sup>, P. Watson<sup>2</sup>.* 1) Exact Sciences, Maynard, MA; 2) Creighton University, Department of Preventive Medicine and Public Health, Omaha, NE.

People with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) have an 80% lifetime risk of developing colorectal cancer (CRC). As these cancers may show accelerated carcinogenesis with early onset, patients are advised to begin colonoscopy screening beginning at the age of 20 to 25 and repeated every one to two years for the rest of their lives. Despite the fact that colonoscopy has shown the ability to reduce the mortality and incidence of CRC in this group, as many as 30% of HNPCC gene carriers do not follow these recommendations. In addition, patients who are compliant with these screening recommendations may still develop interval CRC. A stool based CRC screening test was developed which may have clinical value in the noncompliant patient or between colonoscopy screening years. This test is designed to detect mutations in BAT-26, a marker for microsatellite instability, a common feature in HNPCC colorectal cancers and adenomas.

A total of 47 patients were recruited to participate in this study. Three patients were excluded because they were determined to be mutation negative after their colonoscopy (all three had normal colonoscopies and negative stool tests). Of the remaining 44 patients, three were found to have CRC at colonoscopy (2 Dukes A and 1 Dukes C) and one patient had advanced adenomas as determined by size (a 2-3 cm tubular and a 1 cm hyperplastic). The stool test was positive in one of the patients with CRC (Dukes A) and in the one patient with polyps. The two cancers from patients that were negative by the stool assay were tested for microsatellite instability, and mutations in BAT-26 were present in only one. All 40 patients with negative colonoscopies were negative by the stool assay. This stool assay has shown the ability to detect early stage disease with 100% positive predictive value, and may have use as an adjunct to colonoscopy screening in people with HNPCC.

**Risk-based referral to a clinical cancer genetics program: a study paralleling the real world of non-research covered service.** *S.M. O'Neill<sup>1,2</sup>, V.G. Vogel<sup>1</sup>, E. Feingold<sup>2</sup>, R.E. Ferrell<sup>2</sup>, J.A. Peters<sup>3</sup>, W.S. Rubinstein<sup>4</sup>.* 1) Comprehensive Breast Program and Cancer Genetics Program, UPCI/Magee Womens Hospital, Pittsburgh, PA; 2) Dept. of Human Genetics, University of Pittsburgh, PA; 3) Clinical Genetics Branch, NCI, Rockville, MD; 4) Evanston Northwestern Healthcare, Northwestern University Medical School, Chicago, IL.

Genetic counseling and testing for BRCA mutations is a prototype for other common adult-onset disorders. We assessed uptake of a cancer genetics program (CGP) referral not covered by research funds in 211 subjects given a computerized breast cancer risk assessment (BRA) in the biopsy and treatment clinics of an urban women's hospital. Subjects completed questionnaires assessing psychological status and knowledge and attitudes about breast cancer (BC), cancer risk counseling, and genetic testing. Of 120 women with no previous history of BC undergoing breast biopsy, 63 (53%) were referred to the CGP on the basis of a Gail or Claus model risk <sup>3</sup>twice the population risk. Of 91 BC patients, 43 (47%) were offered referrals for a mutation risk <sup>3</sup>10% based on the Couch, Shattuck-Eidens, Frank, or BRCAPRO statistical models. At 6-18 months post-referral, only 1/63 unaffected, but a greater proportion (13/43; 30%) of affected, women made CGP appointments, and 10 had testing. Of the decliners, 74 were re-interviewed to elicit reasons for lack of follow-up. Main reasons cited by unaffected women included perceived low risk after benign biopsy results, lack of family history, and satisfaction with the BRA. Affected women cited a dissimilar spectrum of reasons: lack of time, sufficient risk information from their physicians and the BRA, and fear of insurance discrimination. High income was the greatest predictor of uptake ( $p = 0.006$ ). "Not wanting anymore tests" was a significant barrier ( $p = 0.03$ ). Most women (81%) said genetic risk assessment should be routine, suggesting annual mammography or OB/GYN visits as the most effective setting. Studies to date have focused on uptake of genetic testing offered to women as part of cost-free research studies. Uptake of referral may be less in the clinical arena and may depend on timing or setting of the referral.



**Application of the Bethesda and Amsterdam Criteria and the Incidence of Identifiable Genetic Hallmarks of HNPCC.** *S.K. Palmer, T.J. Monroe, M.A. Luchtefeld, J. Miller.* Molecular Biology, Spectrum Health, Grand Rapids, MI.

Purpose: Colorectal cancer (CRC) occurring in young patients and those with a strong family history, increases the suspicion of hereditary nonpolyposis colorectal cancer (HNPCC). It is possible patients may not fit established predisposition criteria and still be diagnosed with HNPCC. The priority of this study was to establish a genetic testing program for families in the state of Michigan who are likely to have or who are at increased risk of developing HNPCC. Individuals were enrolled in the study based on accepted Bethesda/Amsterdam criteria and consented to genetic analysis to evaluate current diagnostic testing regimes. Methods: CRC patients less than 40 years of age and/or those with a documented strong family history of CRC and other cancer were initially screened for Microsatellite Instability (MSI) using the 5 recommended Bethesda Consensus loci. DNA extracted from paraffin embedded or fresh frozen tumor was amplified by PCR using fluorescent labeled primers and electrophoresed on an ABI 377 instrument. MSI identified at 2 or more of the 5 loci was recorded as a positive result. MSI+ patients were further characterized by immunohistochemical (IHC) staining. Sequence analysis of hMLH1 and hMSH2 was additionally performed on selected patients meeting Amsterdam, Amsterdam Modified, or Amsterdam Minus Criteria. DNA was extracted from peripheral blood and the complete coding regions of both genes were amplified by PCR. PCR products were sequenced and aligned with those previously published. Results: Tumor analysis was initially attempted from 89 samples of CRC patients age 40 or less. Results were obtained for 86 samples: 17 pts were found to be MSI+. 11 of these were shown to lack expression of MLH1 or MSH2 as shown by Immunohistochemical staining. Sequence analysis of those patients meeting Amsterdam Criteria is in progress. Conclusion: In the less than 40 year old patients with CRC, MSI+ tumors were present in 19.8%. 64.7% of the MSI+ patients were found to have protein abnormalities in hMLH1 or hMSH2 genes. Sequence analysis of hMLH1 and hMSH2 genes will allow further evaluation of our selection criteria and screening methodologies.

**Development of a Pediatric Cardiovascular Genetics Clinic.** *S.D. Fernbach<sup>2</sup>, J.A. Towbin<sup>1</sup>, W.J. Craigen<sup>2</sup>, J.W. Belmont<sup>2</sup>.* 1) Pediatric Cardiology, Texas Children's Hospital, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The Cardiovascular Genetics Clinic at Texas Children's Hospital was formed five years ago to provide diagnostic consultation, genetic counseling and clinical care for patients and their families with congenital cardiovascular disorders. Many children with chronic, complex disorders are seen in multiple subspecialty clinics and it was felt that a multidisciplinary approach would be well received by families. The team consists of a pediatric cardiologist, two geneticists and a genetic nurse. Children are referred for evaluation of Marfan syndrome (n=65), Muscular Dystrophy (n=46), Mitochondrial Myopathy (n=18), Williams (n=13), Noonan (n=12), Turner (n=13), and VCFS/DiGeorge (n=5) syndromes, other Chromosome Abnormalities (n=6), Mucopolysaccharidoses (n=5), Friedreich's Ataxia (n=1), disorders of cardiac muscle, cardiac conduction as well as other conditions (n=32).

Pedigrees are obtained in a telephone interview prior to the clinic visit. An echocardiogram, ECG, and chest x-ray may be ordered on the day of the clinic visit. The family history and results of the tests are discussed, a physical examination is performed and recommendations for additional testing are reviewed. For newly diagnosed children, description of the condition, treatment plans, the genetic etiology, inheritance and recurrence are discussed. When appropriate, print materials about the genetic condition, informative websites and support group information are given. Initially, families are seen frequently to determine the effectiveness of treatment, assess their understanding of the child's disorder, their coping skills and to review the genetic information. Two hundred and sixteen patients were seen in the year 2000 with referrals from cardiologists, geneticists, neurologists, ophthalmologists, pediatricians as well as self referrals.

**Is MTHFR C677T polymorphism a risk factor in Branch retinal vein occlusion (BRVO)?**. *D. Napper<sup>1</sup>, K. Yanamandra<sup>1</sup>, T.F. Thurmon<sup>1</sup>, J.A. Bocchini Jr<sup>1</sup>, R. Dhanireddy<sup>1</sup>, L.M. Kooragayala<sup>2</sup>*. 1) Dept Pediatrics, Louisiana State Univ Med Ctr, Shreveport, LA; 2) Dept Ophthalmology, Louisiana State University Med Ctr, Shreveport, LA.

Methylenetetrahydrofolate reductase (MTHFR), a rate limiting enzyme in the folate metabolism, converts methylenetetrahydrofolate to methyltetrahydrofolate in the remethylation pathway of homocysteine to methionine. A common polymorphism C677T in the MTHFR gene reduces MTHFR enzyme activity causing hyperhomocysteinemia and hypomethylation of DNA, leading to cardiovascular and cerebrovascular diseases in addition to its involvement in the birth defects such as neural tube defects (NTD), Down syndrome, and orofacial clefts, etc. MTHFR C677T, Factor V Leiden G1691A (FVL) and Prothrombin G20210A (PTII) are known to be risk factors in hereditary thrombophilia. Retinal vascular occlusion (RVaO) including both arterial (RAO) and veinal occlusions (RVO), is the most common retinal vascular disorder after diabetic retinopathy. Although MTHFR C677T was reported to be a risk factor in various abnormalities as mentioned earlier, its role in retinal vascular abnormalities was not well studied. Our laboratory has been studying MTHFR C677T, FVL, and PTII polymorphisms in the newborn and pregnant population from Northwest Louisiana for the past several years. Presently we are studying the role of MTHFR C677T polymorphism in the RVaO patients from Northwest Louisiana attending our LSU medical school Retina Clinic. We have studied so far 62 patients with RVaO comprising of 65% African-Americans (AA), 30% Caucasians, and 5% others. There were 48 RVO patients and 14 RAO. Among the RVO, 35 were AA and 10 were Caucasians. Eighteen AA had BRVO and 17 AA had CRVO. Nine Caucasians had BRVO and 1 had CRVO. Two Asians had BRVO and one Hispanic had CRVO. The remaining patients had RAO. Our data revealed that African-American patients with BRVO carry statistically significant two-fold higher C677T heterozygosity compared to the control AA population (Relative Risk 2.42). Further larger studies are needed to confirm these results. Data and statistics from various RVaO patients and from various ethnic groups will be presented.

Program Nr: 1446

**Screening of Factor V Leiden (G1691A), Prothrombin G20210A and Protein C Gene Mutations in Thrombosis Patients.** *T.A. Ulutin, J. Altinisik, H.O. Ates, M. Cengiz, N. Sayhan.* Biomedical Sciences Division, Istanbul University, Istanbul, Turkey.

Thrombotic disease is one of the leading causes of morbidity and mortality in the world. As the development of thrombosis is multifactorial, it is known that numbers of hereditary and acquired factors are involved in this process with different mechanisms. It is also known that impairment of the natural inhibitor mechanism which regulates coagulation system mostly plays an important role in hereditary venous thrombosis. The presence of FV Leiden, Prothrombin G20210A and Protein C mutations were examined in thrombosis patients. FV Leiden (G1691A) and Prothrombin G20210A mutations were screened by using PCR and restriction enzyme digestion methods. Out of 50 thrombosis patients 2 were found to be heterozygous and 1 homozygous for FV Leiden mutations (6%) and two heterozygous for Prothrombin G20210A mutations (4%). Out of 25 healthy subjects, one had Prothrombin G20210A mutation (4%), while none of them had FV Leiden mutations (0%). There wasn't any significant difference between patient and control groups (FV Leiden  $p=0.29$ , Prothrombin G20210A  $p=0.74$ ). When exons (2,3,4,5,6,7,8,9) of the Protein C gene were amplified by polymerase chain reaction (PCR) and analysed by single strand conformational polymorphism (SSCP); one exon 2, four exon 3, one exon 4/5, two exon 7, three exon 8, two exon 9-3', two exon 9-5', a total of nine patients were found to have probable mutations while no sample from the control group had any mutations.

**Development of a high-throughput test for the ACE insertion / deletion polymorphism using primer extension and MALDI-TOF mass spectrometry.** *T. Stewart, M. Zetlmeisl, L. Leppin, C. Rodi, M.J. McGinniss.* Molecular Biology, Sequenom, Inc, San Diego, CA.

The insertion / deletion polymorphism in intron 16 of the angiotensin-converting enzyme (ACE) gene at 17q23 may play a role in cardiovascular disease. The insertion allele contains an alu repeat of 288 bp that results in allelic imbalance in heterozygous samples during PCR amplification using standard forward and reverse primers. We used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and primer extension to assess mass differences in insertion (I) and deletion (D) alleles using a homogenous Mass EXTEND™(hME) assay. Due to allelic imbalance during PCR, the peak height of the I allele in heterozygous samples was approximately 30% of the D allele. Our assay was redesigned using a third insertion-specific PCR primer that in addition to the normal two PCR primers ensures nearly equal amplification of both the deletion and insertion alleles. Amplicon lengths of three sizes were generated (two for the insertion allele and one for the deletion allele). Sequenom's patented MassARRAY™ technology was then used for genotyping 91 normal control DNA samples with resulting genotype frequencies of 23% II, 31% DD, and 46% ID. We also genotyped ten interlaboratory DNA proficiency samples and the results from this study were 100% concordant. Direct measurement of primer extension products with mass spectrometry is highly accurate, cost-effective, efficient and eliminates the need for retyping that may be required to avoid mistyping of ID to DD when using conventional electrophoretic methods.

**Genomic Medicine In Developing Countries: Research, Clinical And Ethical Implications For Hereditary Colorectal Cancer In South Africa.** *R.S. Ramesar<sup>1,2</sup>, U. Algar<sup>2,3</sup>, R. Felix<sup>1,2</sup>, J.A. Gilfillan<sup>1,2</sup>, P.A. Goldberg<sup>2,3</sup>.* 1) Department of Human Genetics; 2) Colorectal Cancer Research Consortium; 3) Surgical Gastroenterology Unit, Grootte Schuur Hospital and University of Cape Town, South Africa.

A strong research programme has been established in South Africa with the aim of identifying the genetic defects underlying familial as well as some sporadic forms of colorectal cancer. The identification of at least 12 new disease-causing mutations in the primary candidate genes, hMLH1 and hMSH2, has led to the implementation of a predictive testing protocol to supplement the clinical management of subjects and family members at risk. The search for additional mutations is being speeded up by new technologies. In a single family/clan which is widely dispersed along the West Coast of South Africa, 203 of 529 individuals are involved in the investigation and management protocol. The identification of a novel mutation in exon 13 of the hMLH1 gene has led to genetic test results being made available to over 100 individuals. This specific change has been detected in at least 11 other apparently sporadic individuals, who were screened because they were under the age of 45 years and had right sided tumors. The finding of a common haplotype around the hMLH1 mutation, confirms a strong founder effect for the disorder along the West Coast of SA. Further genealogical and molecular investigations in just 4 of the 11 'sporadic' subjects has revealed each as being the 'tips of HNPCC icebergs'. The important issues have to do with: 1) when is it most appropriate to embark on the use of genetic testing, 2) the ethical implications of having genetic information but not the means/resources for imparting this information to a widely dispersed at-risk population, 3) whose responsibility is it to recruit subjects for testing on a wide scale, and 4) how best to develop capacity. There are large areas of South Africa which remain underserved for health care, with genetics facilities limited to only a few academic centres. At-risk populations may be recognisable but are widely dispersed. This paper highlights the practical issues around Genomic medicine, and its power for complementing conventional medical practice.

**Mutation screening by DHPLC: the learning curve.** *E.A. Lobos, R.D. Todd.* Dept Psychiatry, Washington Univ Sch Medicine, St Louis, MO.

Denaturing high performance liquid chromatography detects sequence variants via differential elution from a heated column by an acetonitrile gradient of mismatched DNA heteroduplexes formed by PCR amplification of heterozygous DNA. It has been advocated as a highly sensitive, specific and inexpensive method to screen for mutations, but researchers can find it problematic to implement as a new technology in the lab. We have recently applied a strategy using DHPLC mutation/variant detection, in a sample of individuals with attention deficit/hyperactivity disorder (ADHD), for exons 2-8 of the dopamine D2 receptor gene. We used limited sequencing to determine the variant nucleotides causing the different characteristic elution patterns. Primers were designed for robust product formation rather than for minimal variation in melting temperature across the length of the amplicon (as is recommended). Nonetheless, variants were detected for 6 of 7 amplicons, with heterozygous patterns for 3 of them exceeding 40% in the sample. However, a second primer was used to facilitate genotyping for a larger sample that reduced the length and homogenized the melting temperature of the fragment with 3 variant sites. Genotypes of homozygous individuals of unknown type were confirmed by addition of aliquots of PCRs of known alternate homozygotes to the samples, then denaturing, reannealing, and analyzing by DHPLC. A 10 microliter PCR volume was usually adequate for the entire analysis of the sample. During this test case, we have learned some basic tactics for economical and effective use of DHPLC, including robust and consistent PCR product yield, testing of the amplicon for suitability for pooling, manipulating the gradient to minimize elution time and reagent use, quick testing and recognition of optimal oven temperatures for heteroduplex detection, and warning signs of potential problems with amplicons. Applying these basic techniques allows us to effectively use DHPLC for additional studies while continuing efforts to reduce cost and hands-on time required to generate and interpret results.

**CFTR Mutation Screening In Hypertrypsinaemic Newborn In Italy.** *R. Padoan<sup>1</sup>, M. Giunta<sup>2</sup>, A. Bassotti<sup>2</sup>, C. Corbetta<sup>3</sup>, A. Ambrosini<sup>3</sup>, D.A. Coviello<sup>3</sup>, M. Seia<sup>3</sup>.* 1) Pediatrics Dept., Ist.Clinici di Perfezionamento, Milano, MI, ITALY; 2) Pediatrics Dpt., University of Milan, Milano, MI, ITALY; 3) Laboratory Dept., Ist.Clinici di Perfezionamento, Milano, MI, ITALY.

In the last seven years the Guthrie card screening program, performed on 169.000 newborns of Lombardia region population, identified 39 affected neonates and 25 hypertrypsinaemic infants presenting borderline sweat test (chloride value between 30 and 60mmol/L). Neonatal hypertrypsinaemia has been recently associated to the presence of increased frequency of CFTR mutations and IVS8-5T polymorphism. The aim of our study was to assess the incidence of rare CFTR mutations or polymorphisms in infants with neonatal hypertrypsinaemia and with a "borderline" sweat test in the first months of life. An extended analysis of CFTR gene was performed on the 25 hypertrypsinaemic infants, using DGGE technique on 11 out 27 exons, sequencing analysis of splice site polymorphism in intron 8 (TG)mTn and sequencing analysis of DNAs that showed an abnormal DGGE electrophoretic pattern. The results identified a total of 17 different CFTR alleles: eight DF508, four R117H, two G542X, two N1303K, two D1152H, R347P, 2183AA->G, 621+1G->A, 2789+5G->A, R553X, R347H, R117L, V1153E, Y1032C, 711+3G->A. In addition we identified a novel mutation D110N . Furthermore IVS8-(TG)mTn polymorphisms were tested, and the haplotype TG12T5 was identified in seven infants. In total we identified 14 compound heterozygotes (seven with two CFTR mutations, seven with one mutation/TG12T5), 10 subjects with only one mutation identified, whereas in only one patient no gene alterations were found. In conclusion we suggest that a high frequency of CFTR gene mutations is present among hypertrypsinaemic neonates presenting a "borderline" sweat test in the first months of life. Moreover we suggests that some immunoreactive trypsin positive neonates are actually affected by a very mild form of CF, an extended genetic analysis is recommended and furthermore clinical follow up in specialised Centres is needed to diagnose mild or atypical forms of CF.



**Standardization of PCR amplification for fragile X trinucleotide repeat measurements.** *J.P. Jakupciak, C.D. O'Connell, D.A. Atha, K.L. Richie.* Biotechnology, NIST, Gaithersburg, MD.

To provide the clinical diagnostics community with accurate protocols and measurements for the detection of genetic disorders, we have established a quantitative measurement program for trinucleotide repeats associated with human disease. In this study, we have focused on the triplet repeat associated with fragile X syndrome. Five cell lines obtained from the Coriell Cell Repository were analyzed after PCR amplification and size separation. These cell lines were reported to contain CGG repeat elements ranging from 29 to 110 repeats. Our initial measurements focused on measurement variability; a) between slab gel and capillary separation systems, b) inter-lane variability (slab gel), c) inter-gel variability, and d) variability associated with amplification. Samples were run in triplicate for all measurements, and the analysis performed using GeneScan analysis software. The repeat sizes were verified by DNA sequence analyses. The standard deviations for inter-lane measurements in slab-gels ranged from 0.05 to 0.35. There was also little variation in size measurements performed on different gels and among PCR amplifications. The CGG repeat measurements performed by capillary electrophoresis were more precise, with standard deviations ranging from 0.02 to 0.29. The slab gel and CE size measurements were in agreement except for the premutation alleles, which yielded significantly smaller sizes by CE.

**Detecting mosaic FMR-1 (fragile X) mutations: Southern blotting versus monoclonal antibody analysis.** *E.C. Jenkins<sup>1</sup>, S.-Y. Li<sup>1</sup>, X.-L. Yao<sup>1</sup>, S. Lanter<sup>1</sup>, V. Sudhalter<sup>2</sup>, R. Belser<sup>2</sup>, X.-H. Ding<sup>3</sup>, G.E. Houck, Jr<sup>3</sup>, A. Glicksman<sup>3</sup>, C.S. Dobkin<sup>3</sup>, S.L. Nolin<sup>3</sup>, W.T. Brown<sup>3</sup>.* 1) Department of Cytogenetics, New York State Institute for Basic Research in Developmental Disabilities (IBR), Staten Island, NY; 2) Department of Psychology, IBR; 3) Department of Human Genetics, IBR.

It has been hypothesized that mosaic fragile X individuals have a better prognosis. To determine the most reliable method for detecting mosaicism, we compared Southern blotting with probe StB12.3 to monoclonal antibody (mAb) FMR-1 protein (FMRP) detection with 1A1 (Chemicon 2160). Among 48 males who exhibited either full or mosaic FMR-1 mutations following PCR and Southern analyses, the percentages of negatively stained or FMRP-negative small lymphocytes ranged from 66-100. Two hundred cells were analyzed per whole blood specimen. However, there was incomplete concordance between the numbers/percentages of cells that were negatively stained with mAb, and Southern analyses relative to detection of mosaicism. For 23 full mutation specimens, the percentages of negatively stained cells ranged from 98.5-100 (14 specimens were 100% negative). In addition, six full mutations had 97-97.5% negatively stained cells. Among 14 mosaics identified with Southern analysis, the percentages of cells that did not stain with mAb 1A1 ranged from 73-98. Of these 14 specimens, 13 correlated well with 1A1 mAb-negative staining that ranged from 73-94%. However, another five specimens that exhibited 66-94% mAb-negative cells, were found to be full mutations on Southern analysis. It is concluded that Southern blotting may be generally more reliable, but mAb detection may be more sensitive in the detection of mosaicism. This work was supported in part by a grant to Dr. Sudhalter from the March of Dimes Birth Defects Foundation 12-FY980227, and in part by the New York State Office of Mental Retardation and Developmental Disabilities.

**MTHFR C677T polymorphism in pregnancies with Chromosomal Defects.** *K. Yanamandra<sup>1</sup>, I. Gadi<sup>2</sup>, D. Napper<sup>1</sup>, H. Chen<sup>1</sup>, J.A. Bocchini Jr<sup>1</sup>, T.F. Thurmon<sup>1</sup>, R. Dhanireddy<sup>1</sup>.* 1) Dept Pediatrics, LSU Medical Ctr, Shreveport, LA; 2) LabCorp. Research Triangle Park, NC.

The pioneering work of professor Smithells and his colleagues in 1960s and 70s on the periconceptional multivitamin and folic acid supplementation study to prevent Neural tube defects (NTD) have paved the way for the plethora of reports on the association of Hyperhomocysteinemia, folate and its metabolizing enzyme gene polymorphisms such as methylenetetrahydrofolate reductase (MTHFR), Methionine synthase (MS), Methionine synthase reductase (MTRR), and other enzymes, with birth defects such as NTD. Although not confirmed there may be association of MTHFR C677T polymorphism with Orofacial birth defects. Our laboratory has been working on the role of MTHFR C677T in the pregnant and newborn population from Northwest Louisiana for several years. We have for the first time reported a two-fold higher MTHFR 677TT homozygosity in the pregnant women at risk for chromosomal defects identified through our triple screening program and a very high homozygosity in Louisiana Hispanic pregnant women and newborn infants. MTHFR, a rate limiting enzyme in the folic acid metabolism, converts methylenetetrahydrofolate to methyltetrahydrofolate in the remethylation pathway of homocysteine to methionine. A common polymorphism C677T in the MTHFR gene reduces MTHFR enzyme activity causing hyperhomocysteinemia and hypomethylation of DNA, leading to various abnormalities. In the present study, we have collected 52 amniotic fluid specimens with abnormal karyotypes, studied the MTHFR C677T polymorphism, and compared them with matched controls. After data culling, we found a statistically significant 1.5 fold increase in 677TT homozygosity in chromosomally abnormal specimens of Caucasians (relative risk 1.9). Among those with non-Down syndrome abnormalities, there was a significant 1.7 fold increase (relative risk 4.0). Specimens with non-Down syndrome abnormalities were also significantly associated with 677TT more often than those with Down syndrome (relative risk 2.1). Data, statistics and composition of abnormal karyotypes will be presented.

**Rare and New Beta Thalassemia Mutations in Iran.** *K. Kahrizi<sup>1</sup>, A.A Poorfathollah<sup>3</sup>, N. Amirzadeh<sup>1</sup>, T. Jogathaei<sup>1</sup>, M. Neishabury<sup>1</sup>, A. Hatamy<sup>1</sup>, K. Javan<sup>1</sup>, N. Amirzadeh<sup>1</sup>, C. Oberkanins<sup>4</sup>, W. Krugluger<sup>5</sup>, H. Najmabadi<sup>1,2</sup>.* 1) Genetics, Welfare and Rehabilitation Uni, Tehran, Tehran, Iran; 2) Karimi-nejad Pathology and Genetics Center Tehran, Iran; 3) Iran Blood Transfusion Services Tehran, Iran; 4) Vienna Lab, Labrodiagnostika GmbH Vienna, Austria; 5) Institute of Clinical Chemistry, Rudolfstiftung Hospital Vienna, Austria.

Beta-thalassemia is the most common hereditary disease of Iran, with a broad spectrum of mutations and so far only around of 20 mutations being tested. In order to reduce the birth of affected individuals we need to improve our technical ability in terms of mutation detection. This panel only detect about 70% of our population mutation. About 3 years ago we decided to investigate other mutations in our population in order to ease and speed up prenatal diagnosis. Total of 230 DNA samples from un-identified Beta- Thal cases representing different geographical areas and ethnical group were collected. DNA were tested for new mutation which have been previously reported from other neighboring country by new version of Reverse hybridization (Vienna Lab). One hundred of the remaining samples which they were negative for mutation by reverse hybridization were subjected to sequence analysis, 10 known mutations(-88 C-A, -87 C-G,-28 C-A, 5'UTR+22 G-A, cd 24/25 -GGT, cd 25/26 +T, cd 42/43 +T, cd82/83 -G, IVSI-128 T-G, IVSI-130 G-C)were identified, which can be added to this list of the rare B-thalassemia mutations in Iran. These results further characterize the heterogenous spectrum of b-thalassemia mutations in Iran and will improve the abilities in prenatal diagnosis and carrier detection for b-thalassemia in order to prevent this prevalent disease in this country.

**Hereditary haemochromatosis: pilot study of case-finding approach to early diagnosis in primary care.** *J. Emery<sup>1</sup>, P. Rose<sup>2</sup>, J. Harcourt<sup>2</sup>, K. Robson<sup>3</sup>*. 1) General Practice Research Unit, Institute Public Health, Cambridge UK; 2) ICRF General Practice Research Group, University of Oxford; 3) Institute of Molecular Medicine, University of Oxford.

**Introduction:** Hereditary haemochromatosis (HHC) is a disorder of excessive iron absorption associated with a range of diseases and non-specific symptoms. 1 in 250 people of Northern European origin are homozygous for the commonest mutation (C282Y) that causes HHC. If therapeutic phlebotomy is instigated before the onset of cirrhosis or diabetes, a normal life expectancy is achievable. While doubts remain about the cost-effectiveness of population screening, a case-finding approach to early diagnosis has been proposed. **Aims:** To pilot a case-finding approach to early diagnosis of HHC in primary care. **Methods:** During a 4 week period 14 general practitioners in Oxfordshire completed consultation proformas recording the presence of any symptoms or diagnoses associated with HHC. Patients aged 25-70 with at least one of these symptoms were invited to return for a consultation with a research nurse and be tested for HHC. Patients were tested for the two common genetic mutations (C282Y and H63D) associated with HHC and iron studies were conducted on any C282Y homozygotes or compound heterozygotes (C282Y/H63D). **Results:** 4022 consultations occurred in which 169 patients (4.2%; 95% CI 3.6-4.8) were recorded with a symptom or diagnosis associated with HHC. Of these 88 patients (2.2% 95% CI 1.7-2.6) were aged 25-70 of whom 61 agreed to be tested. The commonest symptoms for which testing was offered were arthralgia (33 patients), diabetes (22) and fatigue (16). No C282Y homozygotes or compound heterozygotes were identified. 3 C282Y heterozygotes, 14 H63D heterozygotes and 2 H63D homozygotes (not associated with iron overload) were identified. **Conclusions:** This pilot study suggests that a case-finding approach is unlikely to be a cost-effective method of identifying patients with HHC. We must await the results of epidemiological studies into the proportion of homozygotes and compound heterozygotes that develop significant disease before making decisions about general population screening for HHC.

**New Jersey Fetal Abnormalities Registry (NJFAR): Recruitment of cases with structural fetal abnormalities.** *K. DePrince, A.J. Fisher, D.L. Day-Salvatore, J.C. Smulian.* New Jersey Fetal Abnormalities Registry, UMDNJ-Robert Wood Johnson Medical School/Saint Peter's University Hospital, New Brunswick, NJ.

NJFAR collects information on pregnancies after 14 6/7 weeks gestation in which a fetal structural anomaly has been identified. Cases are submitted anonymously to NJFAR by selected Maternal-Fetal Medicine practices. When verbal permission by the subject is given, she is contacted by NJFAR personnel via telephone or in person to explain the purpose and procedures of the registry. Written informed consent is obtained if the subject agrees to participate in an extensive interview and allow the release of medical records. We report the experience with recruiting subjects to NJFAR at the largest participating practice (UMDNJ-RWJ Medical School/St. Peter's University Hospital) over the first nine months of operation. All potentially eligible cases with fetal structural abnormalities were identified from September 2000 - May 2001. This list was compared to the list of cases actually reported to NJFAR. Cases were reviewed to determine the proportion of subjects offered participation in NJFAR and what happened when offered. There were 92 subjects eligible to participate of whom 42 (46%) were offered participation. Of these, 39 (93%) agreed to be contacted by NJFAR personnel. Of the 32 subjects actually contacted, 25 (78%) gave verbal consent to participate and 17 (53%) have provided informed consent to date. These data show that the majority (78%) of women who are contacted about participation agree to participate in the in the project. However, the largest obstacles to ascertainment and recruitment were under-reporting of eligible cases to NJFAR and subjects not being asked if they would be willing to be contacted for further information on participation. We speculate that the possible explanations for our findings might include: clinical time constraints for practitioners, staff changes, general reluctance to approach patients about a research study at the time of initial diagnosis, disrupted emotional state of a patient, language barriers, and not incorporating the recruitment protocol into routine practice. (Supported by the CDC and NJ Dept. of Health & Senior Services).

**Birth defects in Brazil: an emerging public health issue.** *D.D.G. Horovitz, M.G. Monteiro, J.C. Llerena Jr., R.A. Mattos.* Inst de Medicina Social, Univ Estado do Rio de Janeiro, Rio de Janeiro RJ, Brazil.

The burden of congenital anomalies in infant mortality and in hospital admissions has long been recognized in developed countries. Their impact in developing countries, such as Brazil, is more difficult to precise, due to infant mortality being mostly related to perinatal causes and infections. The main purpose of this research was to review through the Brazilian Ministry of Health's databases (<http://www.datasus.gov.br>), trends regarding infant mortality from 1980 to 1997, as well as hospital-based mortality related to birth defects (BD). BD were the fourth cause of infant mortality in 1980/81 (6%), rising to 12% and to the second cause of death in 1996/97. Perinatal causes placed first throughout the period; however, a decrease in absolute numbers of deaths related to all mortality causes was observed, except for BD, which continued as a steady plateau. Among state financed hospital admissions in year 2000, 4% of hospital deaths in all pediatric patients were directly related to a BD. This data is certainly an underestimate of the high morbidity of BD, for often only the immediate cause of death is registered in death certificates. However, it reinforces the magnitude of BD in Brazil, comprising a growing proportion of infant deaths as other major mortality causes are controlled. This emerging public health issue points to the need of future health policy strategies, in a country where health is a Constitutional right to be provided by the government to all citizens. We present possible solutions for integrating the fragmented available genetic services, mostly university-based and concentrated in large urban areas, to the recently established public health system (SUS, Sistema Unico de Saude) as a functional network. Also, working to implement a newly introduced BD field in the birth certificate may help to establish a national BD registry. Further public health actions contemplate introducing clinical genetics as a medical specialty in the SUS, creating continuous medical education programs and, most important, increasing public awareness towards the importance and preventive measures regarding BD.

**Financial support: FAPERJ.**

**Influence of genetic factors on infant mortality in Israel.** *J. Zlotogora, A. Leventhal, Y. Amitai.* Public Health Services, Ministry of Health, Israel.

The largest groups of Israeli citizens are Jews (81%), Muslim Arabs (15%), Christian Arabs (2%) and Druze (2%). While in the Jewish population consanguinity rates are low, in the non-Jewish population, particular among Muslim Arabs and Druze consanguineous marriages are frequent (25-45%).

Since the creation of the State of Israel in 1948, there has been a very impressive reduction in infant mortality both among Jews and non-Jews, however, an almost two fold difference is consistent during the years. Indeed, between 1996-1999, the infant mortality rate was twice higher among non-Jews than among Jews (9 and 4.4 per 1,000 live births respectively). Analysis of the factors leading to infant mortality during these years demonstrated that for each etiological category such as prematurity, prerinatal causes or infections, the mortality was higher for non-Jews. However, the most significant differences were found in the rate of congenital malformations and or Mendelian diseases which were responsible for almost 60% of the difference between the two populations. During the 4 years of the study the rate of children with malformations was 3.1 time higher among non-Jews than among Jews (3.94 and 1.25 per 1,000 live birth respectively). A Mendelian disease was diagnosed 8.3 times more frequently among the dead non-Jewish infants than among the dead Jewish infants (1.33 and 0.16 per 1,000 live births respectively).

The differences in the infant mortality rates reflect the higher incidence of congenital malformations and genetic disorders in the Arab population and the lower utilization of the possibility to abort an affected fetus by the Muslim Arabs and the Druze. In order to reduce the impact of genetic factors on the mortality and morbidity, a program for primary prevention and genetic counselling in the community has been initiated.



**Public health strategies to reduce birth defects in Malaysia** . *M. Thong*. Dept of Pediatrics, University of Malaya Med Ctr, Kuala Lumpur, Malaysia .

A major birth defect is defined as an abnormality of prenatal origin that if uncorrected or uncorrectable, significantly impairs normal physical or social function or reduce normal life expectancy. The incidence of major birth defects ascertained from a hospital-based registry at the University of Malaya Medical Centre is 1.8% amongst all births above 24 weeks of gestation. Nearly 30% of these babies died soon after birth despite being managed in a tertiary setting. Only 9.7% had a post-mortem. The 3 commonest birth defects were chromosomal disorders, malformations of the central nervous system and cardiovascular system. No significant differences were found with regards to maternal age, social class, consanguinity, maternal fever/infection, gender or racial groups. Risk factors identified for major birth defects were family history of birth defects, adverse past obstetric events, absence of intake of folate prior conception to first trimester of pregnancy, gestational diabetes or insulin dependent diabetes mellitus and poly or oligohydramnios. Logistic regression analysis identified adverse past obstetric history, absence of intake of folate prior conception to first trimester of pregnancy and the presence of poly or oligohydramnios as independent risk factors for birth defects in Malaysian newborns. Presence of hydramnios is a useful marker for birth defects and an indication for the selective use of limited detailed ultrasonography in pregnancy. Our study showed that relatively simple population-based public health strategies such as periconceptional folate supplementation, appropriate genetic counselling, family studies and screening may reduce birth defects in a developing country such as Malaysia.

**Sickle Cell Screening among Pediatric Latino Immigrants.** *P.J. Gergen<sup>1</sup>, S. Murillo<sup>2</sup>, C.J. Macri<sup>3</sup>*. 1) Center for Primary Care, AHRQ, Rockville, MD; 2) Dept of Pediatrics, Howard University, Washington DC; 3) Dept of OB/GYN, Uniformed Services University of the Health Sciences, Bethesda, MD.

Neonatal sickle cell screening is mandated in approximately 42 of the 50 U.S. states. During the last 20 years immigration has increased the percent of Latinos in the U.S. population. There are no generally accepted guidelines for sickle cell screening among immigrants. As most of the patients with sickle cell disease in the U.S. are African American, it is unlikely that the average clinician considers sickle cell screening to be a priority among their non-U.S. born Latino patients. Therefore we reviewed the medical charts of all non-U.S. born Latino children/adolescents seen at an inner city clinic serving a primarily immigrant Latino population. Approximately 50% of the patients came from El Salvador, 13% from Mexico, 13% from Dominican Republic, and the rest were from the remaining Central/South American countries. The patients ranged in age from 1 month to 23 years at the time of screening. Of the 187 charts screened, 4 or 2.1% were positive for sickle cell trait. All positives received appropriate counseling. Three of the positives were from El Salvador and 1 was from Bolivia. Hemoglobin and MCV values were similar between the sickle cell trait positive and negative groups. U.S. neonatal screening data report a prevalence of sickle cell trait of 0.5% among U.S. born Latinos, 7.1% among African Americans, and 0.2% among whites. Clinicians should include sickle cell screening as part of their initial health evaluation of all their non-U.S. born Latino patients.

**Mammography has poor sensitivity in women below 40 years with family history positive breast cancer.** *M.E. Emanuelsson, F. Wiklund, S. Carlson, H. Gronberg.* Dept. of Radiation Sciences, Umea University, Umea, Sweden.

Background; Inherited factors are common in the ethiology of breast cancer in women below age 40. Currently surveillance with breast exam and mammography is recommended to women with a positive family history (FH) of breast cancer. However, the sensitivity of mammography among these women has not been validated in large studies.

Aim; In a population based study of women below age 40 with breast cancer evaluate the sensitivity of mammography with respect to FH.

Patients and methods; All 173 women with breast cancer before age 40 diagnosed between 1980-1990 in northern Sweden were included. Data on family history, stage, grade, mammography result and treatment were prospectively collected. In 136 women information on both FH and mammography result were available. Multiple logistic regressions were used to evaluate which factors that influence the sensitivity of mammography among these women.

Results; In 51 % of these women the breast tumours were not detected on the clinical mammography done before surgery. The mammography negative cases were even more common among FH positive cancers ( 63%). Mammography negative tumours were also smaller (52% vs. 38%, pT < 20 mm) and node-negative (69% vs. 54%, N0). In the multivariate analysis a positive family history (OR=2,46; 1,00-5,93) and tumour size (< 20 mm) were the only 2 factors that independently influenced the detection rate of breast cancer.

Conclusions; In young women with breast cancer (< 40 years) mammography only detected half of the cancers and the detection rate was even significantly less in women with a positive family history. These results clearly indicate that additional screening techniques has to be evaluated particularly in women with positive FH of breast cancer.

**The combined psychoanalyst-geneticist consultation : a 10 year experience.** *C. BRUN, S. Lyonnet, A. Munnich.*  
Genetics, Necker Hospital, Paris, France.

The time when an information is delivered by the geneticist to the patient is crucial and pathetic. Indeed, the highly efficient diagnostic tools of the geneticist are usually regarded as almost magic by the patient, especially because of their speed and efficiency in supplying conclusive diagnoses and verdicts. Since 1991 psychoanalysts attend the genetic consultations at the Department of Genetics, Necker Hospital, Paris. Attending the consultation and observing the situation, we have noted that despite long and most pedagogical explanations, those informations fall on deaf ears as the patient is scared, staggered by recent traumatic events or informations and he understands very little of the expert's explanations. Because he attends the genetic consultation, the psychoanalyst can play a major role at this point. Sitting beside the geneticist who "reads in the future", the psychoanalyst can speak about what is written. This novel form of cooperation is particularly interesting as i) the psychoanalyst is requested by the geneticist at the time and place of the consultation, ii) the geneticist, facing the patients and the family's drama of transmission, is supposed to deliver a major information which not only refers to formal genetics but also to the personal history of the subject. The psychoanalyst attending this long consultation listens to the patient's story, gives meaning to the process and therefore allows time to start again. Because of the traumatic nature of diagnosis, time is often short-circuited, a feature which prevents the investigation from proceeding further. During the genetic consultation, a recognition and reparation process is initiated. Because the present must become livable, reporting his experience and what he has endured helps the patient or the parents facing their future. The psychoanalyst often meets the patient again during further consultations, alone or with the geneticist and long after the diagnosis has been delivered. These combined consultations provide a time and a space for cross talks between the patient, the geneticist, and the family constellation (including grand parents and sibs) and help facing the problem of the disease, of aging, death and of the succession of generations.

**The changing face of a melting pot America; new challenges in genetic screening.** *J.D. Greenberg, S.W. Morton, M. Jamehdor, J. Siegel-Bartelt.* Kaiser Permanente, Los Angeles, CA.

Recommendations for carrier screening for autosomal recessive diseases are largely based on population risks associated with ethnic background. For the first time, the recent year 2000 US census allowed individuals to state multiple ethnic backgrounds. Nationally, only 2.4% of residents reported 2 or more races; however 4.7% of Californians and 21.4% of Hawaiians reported 2 or more races ([www.census.gov](http://www.census.gov)). This census data is limited in that it reflects the ethnic background of individuals of all ages, rather than reproductive couples. To investigate this issue, we retrospectively surveyed the self reported racial heritage in pregnant women (and their partners) attending for positive expanded AFP screening to a single clinic in urban Los Angeles for 1991/92 and 1999/2000.

The incidence of 2 or more races was 70/473 couples(14.8%), and was stable; 32/197(16.2%)in 1991/92 and 38/276(14.8%)in 1999/2000. In 45/473(9.5%) couples, the patient and her spouse had single, but different racial backgrounds. In 18/473(3.8%)of couples, 3 of the 4 fetal grandparents were from the same racial group. 6/473(1.3%)of couples reported complex ethnic background with 3 or more racial groups. One patient reported that she and her spouse had the same dual racial heritage.

Our results indicate that for this diverse urban population, current recommendations for screening based on ethnic background can continue to be utilized in most couples (~85%). The 14.8% of couples stating racial diversity was about 3 times higher than persons (4.9%)reporting 2 or more races in LA county in the 2000 census. Almost all of these couples had a lowered risk for having a child with cystic fibrosis compared to the overall US population risk (except in european/Ashkenazi couples). This data is likely regional. Geneticists concerned with public health planning should consider local population characteristics when planning screening programs, especially given the significantly greater number of couples with racial diversity than suggested by the US census data.

**Nine-year experience of a National Thalassemia Registry and its role in the control of thalassemia major in Singapore.** *I.S. Ng<sup>1</sup>, G.P. Tan<sup>2</sup>, H.Y. Law<sup>1</sup>.* 1) Div Genetics, KK Women's & Children's Hosp, Singapore, Singapore; 2) National Thalassaemia Registry, Singapore.

Thalassemia is the most common genetic disease in Singapore, with an estimated incidence of ~ 3%. Population screening for thalassemia has been advocated since 1988. In 1992, the National Thalassemia Registry was established to register all newly-diagnosed patients with the thalassemia gene and to invite all first-degree relatives and spouses for counseling and subsidised screening. Notification of cases come from all the hospitals, primary and secondary healthcare facilities and hematological laboratories. Patients are informed of the notification and are then contacted to provide pedigree information. Their relatives and spouses are invited to attend a counseling session in the Registry. Couples identified prospectively to be at-risk for a child with thalassemia major are then offered the option of prenatal diagnosis.

The data from the Registry from 1992-2000 shows that this strategy of extended pedigree tracing and screening with the availability of prenatal diagnosis has been effective in significantly decreasing the incidence of thalassemia major in Singapore. On direct invitation, only 1.4% refused counseling and after the counseling session, only 0.1% refused screening. All the couples identified to be at-risk, opted for prenatal diagnosis by chorionic villus sampling. The number of new patients with thalassemia major has decreased from about 10-15 per year to none in the years 1997 and 2000 and only 1 each in 1998 and 1999. The lifetime healthcare costs for each patient with thalassemia major in Singapore has been estimated to be ~\$500,000. The government expenditure to set up the Registry was \$100,000 and the recurrent yearly expenditure for manpower, running costs and subsidies for screening is \$300,000. This expenditure is more than compensated by the healthcare savings from the dramatic control of the disease in Singapore. These savings can also be diverted to optimising the care of the existing patients with thalassemia major.

**The "Pandora" Box of population-based genetic carrier screening.** *K.E. Ormond<sup>1</sup>, C. McMahon<sup>1</sup>, K. DeMarco<sup>1</sup>, M. Fiddler<sup>2</sup>, E. Pergament<sup>1</sup>.* 1) Northwestern University Medical School, Chicago, IL; 2) DePaul University, Chicago, IL.

**Purpose:** Current population-based genetic carrier screening offers a paradigm of simultaneously testing for multiple genetic disorders unrestricted by geography, race and family history, a likely consequence of the Human Genome Project and the increasing sophistication of recombinant DNA technologies. Our center reports on a three-year experience testing 1,000 individuals for Ashkenazi Jewish genetic disorders (AJGD) and 498 individuals for cystic fibrosis (CF). **Method:** A DNA panel of 5 genetic disorders (Tay Sachs, CF, Canavan, Gaucher, Fanconi Anemia) offered to Ashkenazi couples in late 1997 was expanded to 7 (Bloom and Niemann Pick) in April 2000. CF carrier screening was routinely offered to all couples considering prenatal diagnosis. **Results:** Overall, 182 carriers were identified (1 in 9); 7 couples were at reproductive risk (1 in 100); and, 4 individuals were actually homozygous for gene mutations (Gaucher [2]; CF [2]). **Conclusions:** Based on our experience, it is imperative that programs offering carrier screening must consider the process of informed consent, the impact of screening on the individual, family and society, and the logistical issues related to testing, including the sensitivity of testing based on mutations analyzed and patient background. We propose models that should be proactively developed to address: 1) who decides what conditions are included in carrier screening; 2) what factors determine these decisions; 3) facilitation of autonomous patient decision-making; 4) disclosure to third parties; and, 5) development of educational resources for the general public and health care providers.

**Psychosocial impact of predictive testing for myotonic dystrophy.** *C. Prevost<sup>1</sup>, J. Villeneuve<sup>1</sup>, M. Tremblay<sup>2</sup>, S. Veillette<sup>3</sup>, M. Perron<sup>3</sup>, J. Mathieu<sup>1</sup>.* 1) Complexe Hosp de la Sagamie, Chicoutimi, Qc, Canada; 2) Quebec University at Chicoutimi, Chicoutimi, Qc, Canada; 3) Cegep of Jonquiere, Qc, Canada.

In the Saguenay-Lac-Saint-Jean region (Quebec, Canada), a predictive DNA-testing program for myotonic dystrophy type 1 (DM) has been available as a clinical service since 1988. From 1 to 12 years (median, 5 years) after receiving predictive testing, a total of 308 participants (44 carriers and 264 non carriers) answered a questionnaire to determine reasons for testing and recall of test result, to assess their perception of the psychosocial impact of predictive testing and to measure their actual general well-being, self-esteem and psychological distress. The reasons for wanting to be tested were to learn if children are at risk for DM or for reproductive decision making (75%) and to relieve the uncertainty for themselves (17%). The majority of participants (96.1%) remembered correctly their test result. Among carriers, 19% consider themselves in a worse psychological state, 49% are more worried about their future health, 25% feel less satisfaction about life, 25% present a lower self-esteem and 53% are more concerned about their childrens risk. Among non carriers, 36% find themselves in a better psychological situation, 49% are less worried about their future health, 48% feel more satisfaction about life, 20% present a better self-esteem and 85% are reassured about their childrens risk. The actual general well-being, the self-esteem (Rosenberg Self-Esteem Scale) and the psychological status (Psychiatric Symptom Index) are similar in carriers, in non carriers and in the reference (Quebec) population; these results are not influenced by the number of years elapsed since predictive testing was done. All respondents believe that predictive testing should be available for the at-risk population and the vast majority of carrier (95%) and of noncarriers (93%) would recommend the use of predictive testing to their family members.



**Application of genetically based individual approach in help decision making system.** *H.V. Baranova<sup>1, 2</sup>, E. Albuisson<sup>1</sup>, L. Chapy<sup>2</sup>*. 1) Medical Faculty, Univ d' Auvergne, Clermont-Ferrand, France; 2) IPPM - Interactive Predictive Preventive Medicine Society, Luxemburg.

We present highly personalised internet available help decision making system - Expert System (ES) for interpretation of different laboratory test results (cardiovascular risk, fatty acids spectrum, immune analyses, nutrition) and specially predictive genetic testing. Bioinformatic platform of ES makes possible to perform combined precise analysis and prognosis for each individual according to his peculiarities: lifestyle factors, clinical and genetic data.

Genetic part of ES currently includes 56 main metabolic and predisposition genes and HLA typing. Major goal of ES genetic program is education and presentation of genetically based individual approach in modern medicine. Special attention is paid to environment-gene interactions and pharmacogenetics. Result interpretation and explanations are written in flexible and interactive manner. Three versions: short, normal and long, also as the references are available, according to user request. Other advantages of ES are: 1) informatic model, which allows regular product renewal according to recent achievements; 2) ES with it data bank is a helpful tool in applied research for epidemiological studies.

In conclusion, ES provides a user with highly personalised interactive clinicobiological result interpretation, including last validated information, what is in special interest for physicians. ES data bank exploitation can be also useful in applied research.

**Essential genetics education for non-genetics health professionals (E.C. Project GenEd).** *R. Harris<sup>1</sup>, H.J. Harris<sup>2</sup>, K. Challen<sup>1</sup>, L. Ten Kate<sup>3</sup>, J. Schmidtke<sup>4</sup>, I. Nippert<sup>5</sup>, C. Julian Reynier<sup>6</sup>, U. Kristoffersson<sup>7</sup>.* 1) Dept Medicine, Royal Infirmary, Manchester, England; 2) Brooklands Beacon Medical Practice, Manchester, England; 3) Dept Human Genetics, Free University, Amsterdam, Netherlands; 4) Institut fr Humangenetik Medizinische Hochschule, Hannover, Germany; 5) Institut fr Humangenetik, Mnster, Germany; 6) Institut Centre Regional de lutte contre le cancer Paoli-Calmettes, Marseille, France; 7) Dept Clinical Genetics, University Hospital, Lund, Sweden.

We have evaluated the provision of genetic services in 31 European countries and carried out a series of Confidential Enquiries in UK into counselling by non-geneticists. Urgent recommendations included the need to promote genetics education for non-geneticist health care professionals as the welfare of clients and families are threatened by deficiencies in genetic attitudes, skills and knowledge amongst non-geneticists health professionals (including primary care). Our new study of 1879 referrals has identified the main primary care providers who use genetic centres and confirmed that perceived or real risk of hereditary cancer is the harbinger of the rapidly escalating challenge. The European Commission has therefore funded our GenEd study which assesses genetic attitudes, skills and knowledge, collates current policies and practices for educating non-genetics health professionals and identifies deficiencies. The results of the study will provide an empirical base for new educational programs whose aims are to increase understanding of genetic testing amongst non-genetics health professionals and to raise their awareness of consumer issues in the provision of genetic tests.

**Multidisciplinary community hospital based genetic risk assessment program: a model.** *R.R. Lebel<sup>1,2</sup>, D. Sweet<sup>2</sup>, S. Haibeck<sup>2</sup>, M. Maney<sup>2</sup>.* 1) Genetics Services, Glen Ellyn, IL; 2) Hinsdale Hospital, Hinsdale, IL.

Availability of expert genetics consultation has not typically been found outside of university centers, which are often inconveniently located for suburban and rural consultands. To provide such services in a community hospital, we conceived a clinical program housed in a comprehensive oncology department in a large community hospital known for innovative services. The staff includes a clinical geneticist, an oncologist, and oncology specialist nurses trained in obtaining thorough family and health histories. The hospital foundation supplements funding needs which are beyond the income generated by billing for services. During the first year, the focus was on breast cancer genetics. During that time, we also had consultations for other cancers (uterus, colon, pancreas, etc.), as well as other genetic risk factors (hemochromatosis, osteogenesis imperfecta, cystic fibrosis, etc.) We review the clinical experience of the program, its concept and function, with emphasis on interactions with the general medical staff and hospital administration, and other practical aspects of opening and successfully maintaining such a program.

**Primary care physicians' ethical views.** *J.C. Fletcher<sup>1</sup>, D.C. Wertz<sup>2</sup>*. 1) Keswick, VA; 2) Univ. Mass Med. Schl., Shriver Div., Waltham. MA.

We surveyed 900 US primary care physicians (board-certified in obstetrics, pediatrics, or family practice), randomly selected from the AMA Physician Masterfile. 499 returned anonymous questionnaires on ethics. 75% had referred patients to genetic specialists in the past year. We compared responses with those of 529 US doctoral-level geneticists and 555 MS genetic counselors. More primary care physicians than doctoral-level geneticists or counselors would counsel directly after PND for all 21 fetal conditions listed ( $p < .05$ ). For 13 conditions more primary care physicians would urge carrying to term or provide optimistically slanted information. Fewer primary care physicians than genetics professionals would themselves have abortions for 19 conditions. Fewer physicians (63%) than geneticists (84%) or counselors (87%) said women should have unqualified rights to abortion. Fewer (39% v 54% and 66%) would support the decision of a woman with uncontrolled PKU to carry to term, or support a CF carrier couple who want to take their chances (63% v 79% and 96%). More (45% v 25% and 10%) would suggest sterilization to a blind woman on welfare with genetically transmissible blindness. More (55% v 38% and 16%) said it was socially irresponsible to have children with serious disabilities in an era of PND. More (61% v 50% and 57%) would maintain confidentiality of a patient with HD who will not tell relatives at risk. More would test children for predisposition to Alzheimer (58% v 34% and 15%), alcoholism (76% v 52% and 41%), or HD (66% v 37% and 16%). Fewer (28% v 77% and 79%) thought a 16 yr old should be able to refuse tests. More (53% v 33% and 26%) would tell a school a diagnosis of XYY. Fewer (38% v 62% and 65%) would maintain confidentiality of a bus driver with FH at risk of heart attack on the job. Fewer (52% v 75% and 81%) thought workplace screening should be voluntary.. Results suggest wide divergences between primary care physicians and genetic professionals, with the former more directive and less-autonomy oriented, and a need to establish consistency across professions.

**Appropriate compensation for access to individuals' DNA.** *J.C. Bear.* Memorial University, St John's, NF, Canada.

Access to individuals' DNA is now commonly sought by commercial interests intent on using genomic information to develop new drugs for common conditions. The profitability of such products makes it inappropriate to expect individuals to allow DNA access voluntarily and for free, as is usual now. To establish appropriate compensation, it is necessary to estimate the value of information in individuals' DNA. A common scenario illustrates this procedure. In using affected sib pairs to find a locus at which allelic variation has a significant effect on, for example, obesity, in the order of 1000 individuals' DNA may be assessed to obtain the genomic information from which a targeted molecule can be developed. Approximately 10% of targeted molecules become pharmaceutical products. A new drug for the management of obesity would be expected to generate profits in the order of \$1 billion. Thus, on plausible assumptions, profit of \$100,000 relates to the information in the DNA of each individual studied. Because US pharmaceutical firms' returns to investors approach twice those usual for US corporations, and because initiating drug development from genomic data should increase efficiency, a 50/50 division of profit is reasonable between commercial interests utilizing individuals' DNA information and individuals allowing DNA access. Therefore, in this example, a fee of \$50,000 is appropriate for access to an individual's DNA. In addition, because profits may exceed the initial estimate of \$1 billion, a royalty regime is required to distribute an appropriate portion of any additional profit to individuals providing DNA access. Property rights to tissue may be lost once it is removed from the body. Therefore compensation should be contractually secured before DNA access is granted. The approach outlined here for individuals can be adopted by governments (such as Iceland's) or interest groups representing populations of particular genetic interest, to establish total compensation for their collectivities. If multiple disease susceptibilities are to be assessed, a higher DNA access fee should be established. Because access to their citizens or members could be arranged relatively easily, an efficiency premium could appropriately be required.

**Genes in families: attitudes toward testing and treatment options.** *M.E. Coors.* Healthcare Ethics/Human MedGen, Univ Colorado Health Sci Ctr, Denver, CO.

Educational understanding about medical genetics and the risks and benefits involved are a necessary component of informed consent prior to genetic testing. A pilot study conducted at a schizophrenia research center measured baseline knowledge of genetics and the impact that additional education had on attitudes toward testing and treatment. Parents of children diagnosed or at risk for schizophrenia and controls responded to an in-office survey concerning testing for multifactorial genetic disorders and treatment options. Survey results indicated a satisfactory level of general knowledge about genetics for all participants. Subjects and controls indicated a strong preference in favor of genetic testing for adults. Scores also supported genetic testing for at risk children, even in the absence of treatment options. In contrast, subjects and controls were skeptical about pre-emptive treatment following a positive test for disease genes. Both groups preferred postponing treatment until symptoms appear. There were no statistically significant differences between subjects and controls for any of the categories surveyed.

Subjects and controls then viewed an educational power point tool presenting the science and emphasizing the risks involved with genetic testing. Risks included possible insurance or employment discrimination, stigmatization, breach of privacy, and family conflict. Following the educational session, all participants answered the same survey a second time. Second round scores were not significantly different from first round scores. The results of this pilot study reveal that educational information about the risks and benefits involved had almost no impact on attitudes toward genetic testing and treatment. Results indicate that patients and controls want therapeutic benefit and improved pharmaceuticals to result from genetic testing and are less concerned about the possible risks.

**The "Genetic Family"?: The impact and implications of the "Genetic Revolution" on the definition of family.**

*T.A. Caulfield*<sup>1</sup>, *B.M Knoppers*<sup>2</sup>. 1) Health Law Inst, Univ Alberta, Edmonton, AB., Canada; 2) CRDP, University of Montreal, Canada.

In the past few years, there have been a variety of policy statements calling for more oversight of gene tests for a disease predisposition. There has, however, been little formal commentary on paternity testing. Documents such as the Joint Statement of the ASHG and the ACMG on Genetic Testing in Adoption focus on the right of adopting parents to request and access genetic testing data but not on the impact of the current "genetic revolution" on reemphasising the role of biology in the definition of family. Over the past half century, modern family law has emphasized a more inclusive and flexible notion of family. A number of commentators have suggested that the emerging emphasis on genetic relatedness has the potential to narrow the conception of family to those with whom we share a molecular heritage. Given the complex and mutable nature of family relations, a reliance on simplistic notions of biology can hardly be considered a constructive trend. Factors that may be contributing to this trend include the aggressive marketing strategies used by the paternity testing industry, the judiciary's lack of understanding of the role of genetics in human disease, and a growing belief in the "right" to know one's genetic heritage (e.g., Article 7 of the Convention on the Rights of the Child). This paper will: 1) critique the concerns associated with the impact of genetics on family law; 2) overview the evidence to support the concerns (e.g., a recent survey of Canadian paternity testing case law (Caulfield, 2001)); 3) consider the possible negative implications of an over emphasis on genetics in this context (e.g., possible erosion of the significance of the legal principle of the "best interest of the child"); and 4) provide a number of recommendations for consideration.

**Ten years struggle for prenatal diagnosis (ethical social and technical aspect) in Iran.** *T. Khatibi*<sup>1,2</sup>, *R. Karimi-nejad*<sup>3</sup>, *M.H. Karimi-nejad*<sup>3</sup>, *H. Najmabadi*<sup>2,3</sup>. 1) University of Welfare and Rehabilitation Tehran, Iran; 2) UCLA LA,CA; 3) Karimi-nejad Pathology and Genetics Center.

As we have entered the new millennium, Iran is suffering from more than 25,000 affected Beta Thalassemia major, with the variable carrier frequency in different region of the country in some area as high as 1 in every 4 individuals. The Beta-thal preventions started 10 years ago as self motivated and regulated social need in the form of pre-natal diagnosis. Even though Iran is a religious country and therapeutic abortion was totally unacceptable, but according to the need of society the question was raised: how can we prevent un-treatable hereditary disease without sacrificing religious and cultural traditions. Iranian geneticists decided to hold meeting with religious officials to discuss the role of prenatal diagnosis, particularly for B-thal. Finally few years ago religious leader accepted the fact and issued permission to perform therapeutic abortion up to 16 weeks of gestation for B-thal. Simultaneously, the government began to involve and set up systematic approach for control and prevention of the disease. We have also developed a coverage system that insurance companies will cover this kind of medical expenses, have speed up the prevention program. Two national reference laboratories have been approved by government to help prenatal diagnostic program by providing training, problem solving, quality control and quality assurance for themselves and referral laboratories. Fortunately now we have a system which is compatible with our social beliefs. Majority of expenses paid by insurance companies and approved by international agencies like WHO. The effort of the past few years was showing minimal effect on the society; however, as we enter the long phase we should compare Iran's prevention program with those with the other countries such as Cyprus, Greece and etc. We are expecting this year only 2000 prenatal diagnosis will be done. Our system is very efficient and compatible for the region and can be applied as a functional adaptive system for the other Islamic Countries.



**Experiences of serving on a Research Ethics Committee (REC) in the UK: impact of the 'new genetics'.** A.S. Rigby<sup>1,2</sup>, C.J. Taylor<sup>1,2</sup>, K. Khoaz<sup>2</sup>. 1) Sheffield Children's Hosp, Univ Sheffield, Sheffield, England; 2) Sheffield Teaching Hospitals NHS Trust, Sheffield, England.

Members of RECs in the UK have the responsibility of ensuring that medical research is conducted in an ethical manner. To fulfil this role, RECs must engage in reasonable discussion of the ethical issues in each protocol that they review. The completion of the Human Genome Project brings added ethical complexities. Issues such as consent to participate, confidentiality and gene patenting are more frequently seen on the agenda. We carried out a review of the work of our REC to see what issues were prevalent and to see whether the 'new genetics' would impact on our future workload. Between 1997-99 South Sheffield REC registered 1070 protocols. Both hospital (n=566) and university research (n=343) predominated with a smaller number from general practice (n=25). Most protocols were either 'approved with amendments' or 'ratified' by the committee. Only a small number were rejected. Typical diseases brought to the RECs attention included cystic fibrosis, the rheumatic diseases and various types of cancer. The ethical considerations of the committee were diverse. Many of our discussions centred on the collection and storage of DNA, particularly where other family members were involved, and where study sponsors wished to develop a DNA bank for future and often unrelated use. Long term storage of DNA was also an issue. Some multinational companies were keen to develop gene patents and the patients understanding of these issues was neglected by the researchers. Patient information sheets often treated DNA collection from either blood or tissue as an incidental part of the protocol and made little effort to explain the concepts of genetic risk or commercial gains from DNA analysis. The 'new genetics' is likely to have an impact on the future work of RECs. Ethics committee members must have continual training while researchers must learn to address real ethical concerns; both parties must learn to work together. If they are not able to do so, the completion of the Human Genome Project will be like climbing Mount Everest: a great achievement in itself, but of little value to others.

**Attitude toward ethical issues in severe congenital anomaly: a survey among medical personnel in Taiwan. C.**

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To investigate the ethical issues encountered in caring of children with severe congenital anomalies and attitude of medical staffs in end-of-life decision making, we conducted a survey among pediatricians and pediatric nurses from different hospitals in Taiwan. Questionnaire consisted of 57 questions and four case scenarios was used to score the attitude which were either pro-life or quality of life oriented. Our results were based on a total of 382 respondents. The results showed that (1) Withdrawing and withholding life support in these children was considered as the most difficult ethical dilemma by pediatric nurses. (2) Parents' wish was considered as the most important factor in the decision of to treat or not. (3) Staffs who believed in Buddhism and Daoism took attitudes that were more pro-life oriented (31.20+7.36 vs 29.05+4.56;  $P < 0.05$ ). (4) Nurses in ICU tended to take more quality of life attitude than nurses in general wards. We concluded that understanding the psychosocial factors involved in medical decisions for these patients would help in counseling and clinical practice. And a critical self-analysis of medical personnel's behaviors would lead to a decision making process grounded on a more sound ethical basis.

Program Nr: 1477 from the 2001 ASHG Annual Meeting

**Catalyst for Advancing Research: Collaborations among Industry, Academia and Consumer Organizations.** *S.F. Terry<sup>1, 3</sup>, J. Cody<sup>2</sup>, J. Lewis<sup>3</sup>, P.F. Terry<sup>1</sup>, L.M. Nelson<sup>4</sup>, M.E. Davidson<sup>3</sup>*. 1) PXE International, Inc, Sharon, MA; 2) University of Texas Health Science Center, San Antonio, TX; 3) Genetic Alliance, Washington, DC; 4) National Institute of Child Health and Human Development, Bethesda, MD.

As the field of genetics matures, a number of important changes are taking place. The early stages of genetics research involved the development of technology to sequence the human genome. As proteomics takes center stage, research will require collaborations in well-developed networks of researchers, participants and their respective organizations. These partnerships will be both the catalyst and fuel for acceleration of research that is essential to advancing new understandings. In the past academia and industry have often worked on parallel tracks, apart from the lay advocacy and public sectors. However, there are increasing numbers of research collaborations giving rise to effective and efficient interactive models that can sustain the essential resources for genetics research. Borrowing lessons learned from the semiconductor field, and other successful collaborations throughout the research community, the speakers will describe successful model projects and the key elements which guaranteed the partnerships success.

**Community Genetics Carrier Screening for Multiple Disorders.** A. Proos<sup>1,2,4</sup>, H. Aizenberg<sup>2</sup>, K. Barlow-Stewart<sup>2,3</sup>, L. Burnett<sup>1,2,4</sup>, V. Howell<sup>1,4</sup>, E. Raik<sup>4</sup>. 1) Lab and Community Genetics, Kolling Institute, St Leonards, Australia; 2) Institute of Community Genetics, Wolper Jewish Hospital, Trelawney St, Woolahra, Australia; 3) NSW Geneticsw Education Program, Royal North Shore Hospital, St Leonards, Australia; 4) Pacific Laboratory Medicine Services (PaLMS), Royal North Shore Hospital, St Leonards, Australia.

Australia is a highly multi-cultural country, with its current population derived from more than 200 different national and ethnic groups. We have conducted an eight year study to assess the relative effectiveness of different strategies for introducing community genetics screening programs for asymptomatic genetics carrier detection. Here we report the successful extension of these programs to include three simultaneous genetic disorders: Tay-Sachs disease (TSD), cystic fibrosis (CF), and Thalassaemia (Thal). Methods Subjects consisted of senior high school students, 16 years plus. A genetics education session was organised at school in group-delivery mode, describing principles of genetics inheritance, and clinical significance of the three different genetic disorders. Subjects were provided with brief written material and were encouraged to discuss this material with their family and peers. One week later, confidential access to free laboratory testing (HEXA enzyme activity, CFTR mutation detection, automated Red Cell indices/Blood Film examination) for the disorders was offered to students at school. Informed consent was obtained and individual counselling was available. Results. 67% of subjects elected to proceed onto testing. 50 % of subjects elected to have testing for all three disorders even though 18% of these were low risk for TSD + CF. 4.7 % of subjects tested required genetics counselling when their test results revealed that they were an asymptomatic mutation carrier. 0.8 % of subjects required medical consultation when their haematology test results revealed abnormalities. Conclusion. Test selection although based on ancestry and risk was also influenced by availability. Laboratory protocols for community genetics screening for multiple disorders are feasible.

**The Alfred & Baker Gene Bank - A Cardiovascular Genetic Research Investment.** *C. Williams<sup>1</sup>, A. Dart<sup>1</sup>, C. Reid<sup>2</sup>, G. Jennings<sup>1</sup>.* 1) Cardiology Department, Alfred & Baker Medical Unit, Melbourne, Australia; 2) ANBP II, Alfred & Baker Medical Unit, Melbourne, Australia.

**Background:** Genetic mutations have been proposed to be the underlying cause of hypertension, hyperlipidaemia and coronary heart disease in addition to determining individual responses to drug therapy. Establishing a mechanism through which populations, diseased and healthy, can be readily genotyped however, raises logistic and ethical challenges. Our aim was to establish a cardiovascular (CVD) gene bank for healthy volunteers and individuals with cardiovascular disorders to enable the study of the genetic determinants of CVD.

**Methods:** Individuals with CVD attending the cardiology and cardiac surgery departments of The Alfred are routinely approached to provide a blood sample. Healthy volunteers attending risk evaluation services are similarly approached. Subjects are asked to consent for their sample to be available for genetic analysis. The Alfred Research & Ethics Unit was approached to approve a process whereby consent for genotyping of samples rests with the Ethics Committee rather than having to gain consent from each volunteer for every proposed research project.

**Results:** Following external review from the Victorian Council of Bio-ethics, The Alfred Research & Ethics Committee agreed: a) the gene bank could collect samples for genetic analysis in cardiovascular and clinical research projects; b) samples could be stored indefinitely; c) future analysis of samples would be subject to further ethics applications related to the specific genetic study. Subject involvement has been exceptional and few have expressed reservations about contributing. Less than one in 100 participants approached have declined joining the gene bank. Those who refused did so due to concerns relating to privacy laws. In the first 12 months 700 patients have consented to participate.

**Conclusions:** The Alfred & Baker Gene Bank has been established to provide a resource for ongoing research into the genetic determinants of CVD. Authority for the ongoing use of the samples in future is vested in The Alfred Hospital Research & Ethics Committee.

**Evidence for involvement of the *HFE* S65C Variant in Hereditary Hemochromatosis: Implications for Diagnostic Testing.** *I.M. Buysse*<sup>1,2</sup>, *A. Bartlett*<sup>1,2</sup>, *D.W. Stockton*<sup>1</sup>, *B.B. Roa*<sup>1,2</sup>. 1) Baylor DNA Diagnostic Laboratory; 2) Department of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Hereditary hemochromatosis (HH) is an autosomal recessive disorder characterized by increased iron absorption and iron storage leading to organ damage. Clinical consequences can be prevented by early diagnosis and treatment by phlebotomy. HH in the Caucasian population is associated with two major mutations in the *HFE* gene which are assayed in most diagnostic laboratories. Homozygosity for the severe C282Y mutation accounts for >80% of cases, while compound heterozygosity for H63D and C282Y is associated with HH at a reduced penetrance. A third variant, S65C, was also reported to contribute to HH. We describe one patient with a positive family history and a clinical diagnosis of mild HH, who presented with a transferrin saturation level of 39%, a ferritin level of 512, and elevated iron stores on liver biopsy. This patient was initially tested for H63D and C282Y using ASO hybridization, and found to be heterozygous for C282Y. Further sequence analysis identified him to be compound heterozygous for S65C/C282Y. These alleles appear to be in trans, since a first degree relative was reported as S65C/-. We extended the S65C analysis to a cohort of patients who were previously referred for HH testing. An automated primer extension-based assay, Pyrosequencing, was used in our initial survey for S65C which identified 11 positives out of 181 HH patients (~3% of chromosomes). This group included 7 S65C/C282Y compound heterozygotes out of 81 patients previously tested to be C282Y/-; 2 H63D/S65C compound heterozygotes out of 70 who had tested H63D/-; and 2 S65C heterozygotes out of 30 who had tested negative for H63D and C282Y. Analysis of 90 control individuals identified 1 heterozygote and 1 homozygote for S65C (~1.7% of control chromosomes). Our results are consistent with previous reports that suggest S65C allele enrichment in HH patients, and an apparent risk conferred by the S65C/C282Y compound heterozygous *HFE* genotype. These collective findings raise the possibility of including S65C together with C282Y and H63D in a molecular diagnostic panel for hereditary hemochromatosis.

**Molecular analysis of Duchenne and Becker muscular dystrophy patients in Saudi Arabia.** *R. Majumdar<sup>1</sup>, M. Al Jumah<sup>2</sup>, S. Al Rajeh<sup>3</sup>, E. Chaves-Carballo<sup>4</sup>, M.M. Salih<sup>5</sup>, A. Awada<sup>2</sup>, S. Shahwan<sup>6</sup>, S. Al Uthaim<sup>1</sup>.* 1) Neurogenetics Laboratory, Department of Medicine, King Fahad Natl Guard Hosp, Riyadh, Saudi Arabia; 2) Division of Neurology, Department of of Medicine, King Fahad Natl Guard Hosp, Riyadh, Saudi Arabia; 3) Division of Neurology, King Saud University, Riyadh, Saudi Arabia; 4) Department of Neurosciences, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; 5) Department of Pediatrics, King Saud University, Riyadh, Saudi Arabia; 6) Department of Pediatrics, Military Hospital, Riyadh, Saudi Arabia.

**Objective:** The deletion in the dystrophin gene has been reported for many ethnic groups, but until now the mutations in this gene have not been thoroughly investigated in Saudi Duchenne and Becker muscular dystrophy (DMD/BMD) patients. **Methods:** We examined the deletion pattern in the dystrophin gene of the Saudi patients applying multiplex-polymerase chain reaction (PCR). Genomic DNA was isolated from twenty two patients with DMD/BMD confirmed by dystrophin staining on muscle biopsy, eleven patients with clinical suspicion of DMD without muscle biopsy, three patients with limb girdle muscular dystrophy, twelve relatives of the patients, and five healthy Saudi volunteers. Specific exons around the deletion prone regions (hot spots) of the dystrophin gene were amplified. **Results:** The deletion of one or more exons was found in sixteen of twenty two DMD/BMD patients. The deletion in the gene was detected in seven of eleven patients with suspected DMD diagnosis, but not confirmed by dystrophin staining of muscle biopsy. No deletion in the dystrophin gene was detected in control Saudi volunteers, the limb girdle dystrophy patients, and the relatives of patients, as expected. **Conclusion:** The present study suggests that intragenic dystrophin gene deletions (70%) occur with the same frequency in Saudi patients compared with other ethnic groups.

(This study was supported by King Fahad National Guard Hospital and a grant from King Abdulaziz City for Science and Technology, Project AT-18-03).

**An unusual pedigree illustrating the dilemmas posed by screening for the cystic fibrosis mutation R117H.** *J.P. Warner<sup>1</sup>, A.J. Gilfillan<sup>1</sup>, D.J. Porteous<sup>2</sup>, D.R. Fitzpatrick<sup>1</sup>, M.E. Porteous<sup>1</sup>*. 1) Clinical Genetics Service, Molecular Medicine Centre, Western General Hosp, Edinburgh, Scotland; 2) Medical Genetics Section, University of Edinburgh, Molecular Medicine Centre, Western General Hosp, Edinburgh, Scotland.

Antenatal screening for cystic fibrosis (CF) mutations has been available in Edinburgh as a first trimester test since 1990. To date a total of 44000 couples have been tested. In parallel with the screening programme the regional DNA laboratory has been testing proven and suspected CF families and also CBAVD men from infertility clinics. Of 119 R117H carriers identified by the antenatal screening programme 393 were shown to be in cis with the 7T intron 8 variant (R117H 7T). All cystic fibrosis patients tested by the DNA lab over the same period (n=10) with the R117H/DF508 genotype have been shown to have the R117H mutation in cis with the 5T variant (R117H 5T). Of 38 men with CBAVD referred by the regional infertility clinic 3 have the R117H 7T/DF508 genotype which is lower than might be expected. Over the past seven years our interpretation of the R117H 7T/DF508 genotype has changed and we show how the advice given to three couples from a single extended family has changed over time. Given the counselling difficulty associated with predicting a phenotype for R117H 7T/DF508 and the 4 fold excess of R117H 7T over R117H 5T in our population the decision was made in Nov 2000 to remove this mutation from the CF antenatal screening set. On the basis of the handling of this and other families we disagree with the recommendation of the American College of Medical Genetics in their guidelines for population based CF carrier screening (1) that R117H be included in a CF screening mutation set. We believe R117H is not appropriate as a screening mutation and that obtaining fully informed consent for testing is impossible during pregnancy for this mutation. (1) Grody et. al. (2001) Genetics in Medicine vol 3 No.2 pp 149-154.



**Real-time quantitative PCR analysis for alpha-thalassemia-1 of Southeast Asian type deletion in Taiwan.** *D.C. Chu<sup>1</sup>, C.H. Lee<sup>2</sup>, S.W. Cheng<sup>2</sup>, M.H. Lin<sup>1</sup>, D.T. Chiu<sup>1</sup>, T.L. Wu<sup>2</sup>, K.C. Tsao<sup>2</sup>, C.F. Sun<sup>2</sup>, J.D. Liu<sup>3</sup>.* 1) School of Medical Technology, Chang Gung University, Tao Yuan, Taiwan; 2) Department of Clinical Pathology, Chang Gung Medical Center, Lin-Kou, Taiwan; 3) Department of OB/GYN, Chang Gung Memorial hospital, Taipei, Taiwan.

Since homozygosity of the alpha-thalassemia-1 of Southeast Asian (SEA) type deletion results in hydrops fetalis, a novel protocol based on the fluorescent probe involved real-time quantitative polymerase chain reaction (PCR) technique to quantify the relative ratio of intact and aberrant alpha-globin genes in cases with reduced mean corpuscular volume (MCV) has been developed to rapidly screen SEA type deletion in Taiwan. A single TaqMan probe was designed to hybridize both the PCR products of normal alpha-globin gene alleles and the alleles with SEA deletion to ensure identical probe binding efficiency. The ratio of the normal alpha-globin genes to genes bearing the SEA deletion present in each subject was thus determined and expressed in cycle threshold (CT) values. Theoretically, a relative ratio of one to one (normal alpha-globin gene allele / SEA deletion bearing allele) was anticipated in individuals carrying the SEA deletion. Forty-five samples (25 heterozygotes for the SEA deletion and 20 with normal alpha-globin gene alleles) were retrieved from the DNA Study Sample Bank and analyzed retrospectively with this protocol. Data showed that the CT values for the intact alpha-globin gene allele and the allele bearing the SEA deletion in heterozygous individuals were determined to be  $28.74 \pm 1.49$ , and  $26.46 \pm 2.05$ , respectively. Therefore, the ratio of normal alpha-globin gene allele to SEA deletion bearing allele in the carriers was  $1.09 \pm 0.043$ . The CT values for intact alpha-globin gene alleles in normal subjects were determined to be  $28.28 \pm 2.11$ . No ambiguous results were observed from other common aberrant genotypes associated with alpha-thalassemia such as the Philippine type deletion. Based on the results, we concluded that this protocol could provide a rapid approach to mass screen carriers with alpha-thalassemia-1 of SEA type deletion effectively.

**Gastroschisis pregnancies from Northwest Louisiana.** *T.F. Thurmon, K. Yanamandra, D. Napper, R. Dhanireddy, J.A. Bocchini Jr.* Dept Pediatrics-Genetics, Louisiana State Univ, Shreveport, LA.

The Biochemical Genetics laboratory in the department of Pediatrics at LSU medical school, Shreveport, Louisiana, has been screening pregnant women for NTD and Down syndrome defects since 1995 using triple analyte screening. Maternal serum screening results of elevated AFP were confirmed by testing for AFP in the amniotic fluids and by amniotic fluid acetylcholinesterase (ACHE) gel assay in the detection of NTD, Gastroschisis, omphalocele, etc. When we analyzed our elevated Amniotic fluid AFPs and positive ACHE results we found a large proportion of Gastroschisis in the single disorder entities. We found 17 pregnancies with Gastroschisis from the amniotic fluids that were referred to our laboratory for AFP testing. On ethnic stratification we found three African-American patients out of 785 referrals (0.38%), and fourteen Caucasian patients out of 1243 referrals (1.13%). When we checked the characteristics of Gastroschisis pregnancies we found that majority of them were young mothers (over 50% were below 20 years of age at LMP, relative risk of 3.3). No Gastroschisis was detected in the maternal age over 35 years (AMA cases). The season of conception made a difference. The majority of Gastroschisis cases were from the spring conceptions (Jan through April) comprising 60% of the defects when compared to the 33% of normal pregnancies (relative risk of 1.7). Although the cause of Gastroschisis formation is not known it is believed to be due to a vascular disturbance in the omphalomesenteric artery. Gastroschisis being a multifactorial disease, many reports explored environmental status of the pregnancies such as smoking, use of illicit drugs, poor nutrition, teenage pregnancy etc, however, no consensus was reached. Young age and concentration of LMPs in one season in our data are puzzling. Data and statistics will be presented.

**Down Syndrome Screening in *In Vitro* Fertilization Pregnancies: Is there a need for a Human Chorionic Gonadotropin adjustment factor?** L. Rouse<sup>1,3</sup>, T. Marini<sup>1</sup>, D. Grow<sup>2</sup>, P. Pekow<sup>3</sup>, P. Nasca<sup>3</sup>, R. Naeem<sup>1</sup>. 1)

Department of Pathology, Baystate Medical Center, Springfield, MA; 2) Department of OB/GYN, Baystate Medical Center, Springfield, MA; 3) University of Massachusetts School of Public Health and Health Sciences, Amherst, MA.

**Background** Methods for screening Down syndrome during pregnancy involve the measurement of maternal serum (MS) alpha-fetoprotein, MS unconjugated estriol, and MS human chorionic gonadotropin (hCG). Previous studies have suggested that due to increased hCG levels in *in vitro* fertilization (IVF) pregnancies an adjustment factor might be needed. We present the largest series to date studying the levels of hCG during IVF pregnancies and its relationship with the screening outcome using naturally conceived pregnancies as a comparison group. **Methods** The study population consisted of pregnant women with singleton pregnancies during the 5-year study period at Baystate Medical Center with a maternal serum screen (MSS) performed at 15-22.9 weeks of pregnancy. The IVF(exposed) group consisted of all positive singleton pregnancies recorded during the first ultrasound at 5 to 7 weeks after IVF (n=72). The comparison (unexposed) group was selected among naturally conceived pregnancies and matched to the study group at a rate of 3:1 within 2 years of maternal age, and date of MSS (n=216). **Results** Bivariate analyses indicated that the exposed and the unexposed were equally to have statistically similar hCG levels at the time of MSS. An odds ratio of 1.02 with 95% confidence intervals of 0.56-1.85 was calculated for increased hCG levels with a cut-point of 1.22 MoM (Multiples of the Median)(66th percentile) at the time of MSS. The specificity of MSS in the IVF pregnancies equaled 87.5%, and the false-positive level was 12.5%. The specificity of maternal serum screening in the naturally conceived pregnancies was 80.1% and the false-positive level was 19.9%. **Conclusion** Study results indicated that IVF status does not influence the likelihood to have increased levels of hCG when compared with naturally conceived pregnancies at time of MSS, therefore an adjustment factor for hCG levels in the Down's syndrome algorithm may be unnecessary.

**Validity of the analysis of FMRP expression in blood smears as a screening method for the fragile X syndrome in Indian population.** *Z.M. Patel, S.R. Menon, A.K. D'souza, R.A. Adhia, D.A. Sanghavi, H.M. Gawde.* Inst Res Reprod/Genetic Res Ct, Indian Council Medical Res, Mumbai, india.

The fragile X syndrome (FXS) is the leading cause of inherited mental retardation, affecting 1:4000 males in the general population. Characteristic phenotype findings include long face, large ears, macro-orchidism & connective tissue anomalies. Its diagnosis is currently based on the presence of an abnormal cgg repeat expansion at the first exon of the FXS gene (FMR1) which causes the shut down of transcription and subsequent absence of the FXS protein (FMRP). Currently detection of patients and carriers is routinely done by DNA analysis of the cgg repeat. In 1995 an FMRP antibody test was developed to detect the presence of FMRP in lymphocytes from blood smears. Subsequently a pilot study in Netherland proved that amongst males FMRP test was a useful screening method for the FXS. We have tested the validity of the FMRP test to screen males for FXS in India. We studied 90 males with mental retardation of unknown etiology, and 10 normal (males & females). All patients were clinically evaluated and tested for FMR1 mutation (southern blot & PCR). The FMRP test detected 3 patients with full mutation. Males with FXS had an average FMRP expression of 11% (0-21%). Whereas normal males and females had FMRP expression around 86% (64-100%). These results support that the analysis of FMRP expression in blood smears is a valid and simple method to screen males with mental retardation of unknown etiology.

**Rett syndrome in the Israeli population.** *R. Shomrat<sup>1</sup>, T. Naiman<sup>1</sup>, H. Wolf<sup>1</sup>, Y. Yaron<sup>1,2</sup>, A. Orr-Urtreger<sup>1,2</sup>.* 1) Genetic institute, Tel- Aviv Sourasky medical center , Tel Aviv, Israel; 2) Sakler school of medicine, Tel Aviv University, Tel Aviv, Israel.

Rett syndrome (RTT) is a neurodevelopmental disorder which almost exclusively affects females and is characterized by cognitive deterioration with autistic features, loss of acquired skills, stereotypic hand movements, ataxia and epilepsy. Milder variants have also been reported. It is an X-linked dominant disorder that affects 1 in 10 000 to 15 000 females. In most classical cases, mutations have been detected in the MECP2 gene located on Xq28. Most cases are sporadic with de novo mutations. Although presumed to be lethal in males, a few rare cases of males with MECP2 mutations have been described.

Our laboratory provides a genetic molecular diagnostic service for RTT patients and families in Israel. We have, so far tested 26 families using direct sequencing of the entire coding region of the MECP2 gene. The patients were from various Jewish ethnic groups and from Arabic origin.

Of the 26 patients evaluated, only 12 had classical manifestations of RTT, the other 14 patients lacked the full classical RTT diagnostic criteria. Mutation analysis revealed mutations in 11 of the 12 classical cases (91%). No mutation was detected in the other non classical cases. Of the 11 patients with mutations in the MECP2 gene 8 different mutations were found, including 2 insertion mutations that have not been previously described; 2 mutations were located in the methyl CpG binding domain (MBD) and 3 in the repression domain (TRD). 6 mutations were nonsense mutations and 2 were missense. No significant difference of mutation type was noted in the different ethnic groups. Of the 11 cases with mutations 10 were de novo. The familial case consisted of a girl and her brother with severe encephalopathy shared the same 754insC mutation while their mother had no mutation in her peripheral blood leukocyte. This case demonstrates gonadal mosaicism.

**Distribution of normal, intermediate and premutation-size of the FMR1 alleles in a normal population at Risaralda Colombia, SA.** *L. Rengifo, A. Alegria, D. Gaviria, E. Aguilar, J. Rodriguez and Centro de Biología Molecular y Biotecnología de la Universidad Tecnológica de Pereira.* Basic Sci, Fac Med, Univ Tecnológica de Pereira, Pereira, Colombia.

The Fragile-X syndrome constitutes one of the most prevalent hereditary entities causing mental retardation and other behaviour disorders. It is characterized on the molecular level by the expansion of the 5-(CGG)<sub>n</sub>-3 repeat in the untranslated 5' region of the gene FMR1 mapped in the Xq27.3 sensitive folate site. Individuals affected present a characteristic phenotype and cognitive disorders. In order to find the distribution of normal, intermediate and premutation-size FMR1 alleles, a molecular characterization of the FRAXA site was made on 1171 alleles corresponding to 728 X chromosomes of 364 women and 443 men X chromosomes, all normal individuals from 11 towns in Risaralda, Colombia SA. The methods used were PCR and Southern blot techniques. The size of the most frequent allele was 30 repeats of the triplet CGG, in 932 cases (79.6%), followed by the alleles with 20 repeats with 60 cases (5.12%) and by the alleles with 28 repeats with 39 cases (3.33%). The frequency of the normal alleles, between 10 and 40 CGG repeats, was 98% of all individuals (98.2% for men and 97.7% for women). The premutation state appeared in three individuals of the general population (three women), corresponding to 0.37%. For the department of Risaralda, a prevalence of 1/270 was observed. Taking into account that the racial and ethnic composition of this department is typical for the country, these results could be extended to other parts of Colombia.

**Scanning of the whole HFE coding region in HC probands with an incomplete HFE genotype by use of Denaturing High Performance Liquid Chromatography (DHPLC):**. *G. Le Gac*<sup>1,3</sup>, *C. Mura*<sup>2,3</sup>, *C. Ferec*<sup>1,3</sup>. 1) Genetics, Etablissement Francais du Sang-Bretagne; 2) Universite de Bretagne Occidentale; 3) INSERM (EMI 01-15), Brest, France.

Background: Genetic testing for the two common HFE gene mutations, C282Y and H63D, plays an important role both in the diagnosis of the hereditary haemochromatosis (HC) and in the detection of patients at risk of iron overload. However, depending on the population studied, between 4 and 35% of HC probands have an incomplete HFE genotype (they are C282Y or H63D heterozygotes or non-carrier of C282Y or H63D). Based on the recent description of sixteen new mutations within the HFE gene, one may consider of particular interest to scan the whole HFE coding region in this group of HC probands to define if they carry uncommon HFE mutations. The aim of our study was to evaluate the Denaturing High Performance Liquid Chromatography (DHPLC) to set up a rapid and economical screening assay of the complete HFE coding region. Methods: The analysis conditions of each coding exon were carefully worked by a combination of computer-based melting profile predictions and empirical data that used available or specifically created positive controls. Results: We detected all known HFE mutations. Moreover, each melting domain of the HFE coding region without known mutation was investigated with, at least, one polymorphism as reference. As a consequence, DHPLC also gives a high probability of detecting unknown HFE mutations. Thus, scanning of the complete HFE gene in 54 HC probands from Brittany allowed us to find two new synonymous mutations, P303P (903 G->A) and G334G (1002 T->C), and a novel missense mutation, V295A (884 T->C), which is also considered to be a polymorphism. In addition, the amino-acid 295 of the HFE protein is not conserved between species: in humans amino-acid 295 is a valine, whereas in other species like mouse and rats it is an alanine. Conclusion: The DHPLC method can be used for an efficient scanning of the HFE gene in HC probands with, at least, one chromosome lacking both the C282Y and H63D mutations.

**Screening For Metabolic Disorders in Genetic Isolates in Manitoba: New Frontiers! New Challenges!** *C. Prasad, C.R. Greenberg.* Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg, MB., Canada.

We describe our experience with the delivery of genetic services for three rare autosomal recessive metabolic disorders, hepatic CPT1 deficiency (CPT1A), sulfite oxidase deficiency (SUOX) and glutaric acidemia type 1 (GA1) in Manitoba. CPT1A occurs in the Hutterite community with a carrier frequency of 1 in 16. A pilot DNA based newborn screening program for a unique CPT1A Gly 710 to Glu (G710E) mutation has been offered to this community for 16 months, with one affected baby diagnosed. The prognosis is good with presymptomatic detection. The exceptional acceptance in this group has been made possible by direct liaison with a community representative and educational programs. A common 1347-1350delTTGT mutation has been identified to cause SUOX deficiency, a severe neurodegenerative disorder in a remote aboriginal community of Northern Manitoba with a population of 800 and a birth rate of 15/year. Three affected babies have been identified over past 10 years suggesting a high carrier frequency. An educational program on the genetic basis and the availability of carrier testing using video links, direct liaison with local health care providers and community members is planned. DNA-based newborn screening for the single homozygous mutation (GCDH IVS-1 +5g@t) that causes GA1 in the Oji-Cree of northeastern Manitoba has been ongoing for last 2 years. Four affected newborns have been identified. Despite early detection and treatment, prognosis for neurological outcome is suboptimal.

Numerous challenges need to be overcome when a screening program is offered to a particular community. Stigmatization, genetic discrimination, social constraints and lack of information about genetics often act as barriers to community and individual participation. Efforts to educate the community are paramount to the ultimate acceptance of a newborn or carrier-screening program. We discuss strategies that have made CPT1A newborn screening a success within the Hutterite community, and identify challenges with implementation of similar programs in the aboriginal communities of Northern Manitoba.



**Molecular analysis in newborns from Texas affected with Galactosemia.** *Y.-P. Yang, N. Corley, J. Garcia-Heras.*  
Genetic Testing Ctr, Texas Dept Hlth, Denton, TX.

We present data about the spectrum of mutations in the Galactose-1-phosphate uridyl transferase (GALT) gene in 39 galactosemia patients who were identified by the Texas Newborn Screening Program. There were 11 cases of classic type and 28 of Duarte-2. Twenty-four patients were White, 12 were Hispanic, 1 was Black, 1 was Asian and 1 was of unknown ethnic origin.

Blinded studies were done by DNA automatic sequencing of the 11 exons and all the exon-intron boundaries of the gene. Thirteen different mutations including 10 missense mutations, 2 nonsense mutations and 1 splicing mutation were detected in 74 of the 78 mutant alleles (diagnostic efficiency of 95%). The prevalent mutations were N314D (38%), Q188R (36%), K285N (5%), and IVS2nt-2a->g (4%). The other less frequent mutations were S135L and T138M (3% each), T23A, H184Q, Y251S, L195P, Q207X, L264X, and A345D (1% each). Three novel mutations, T23A, Q207X, and A345D, were identified. N314D was in linkage disequilibrium with the three intronic polymorphisms IVS4nt-27g->c, IVS5nt-24g->a, and IVS5nt+62g->a and the 5UTR deletion c.-119->-116delGTCA in Duarte-2 type. The 3 individuals with IVS2nt-2a->g were Hispanic, suggesting it is distinctive of Hispanics because it was not observed previously in other populations. S135L, a prevalent mutation in Blacks, was present in 2 Hispanic patients (genotypes Q188R/S135L and IVS2nt-2a->g/S135L). This finding may reflect a racial admixture or an independent origin of this mutation in Hispanics.

Most patients carried two different mutations (30/39). Four patients were homozygous for Q188R. Only 1 mutant allele was detected in 2 cases (1 classic and 1 Duarte-2 type) and no mutations were found in 1 Duarte-2 patient. Two unusual genotypes were observed in 2 patients homozygous for the Duarte-2 N314D allele and heterozygous for another novel mutation. One case was diagnosed as classic galactosemia (N314D+Q207X/N314D) while another one was diagnosed as Duarte-2 galactosemia (N314D+T23A/N314D).

**Choosing a novel strategy for point mutation detection in the dystrophin gene : DHPLC or BESS ? A preliminary comparison.** *S. Chambert, C. Saquet, M. Claustres, S. Tuffery-Giraud.* Lab Genetique Moleculaire, CHU montpellier, France.

In Duchenne and Becker Muscular Dystrophy (D/BMD), one third of the mutations are not detectable as intragenic deletions or duplications of the dystrophin gene. Because full gene sequencing (79 exons) would be laborious and time-consuming in order to identify point mutations occurring in this gene, we used an approach based on the analysis of dystrophin transcripts (RT-PCR) coupled with the protein truncation test (PTT) during these last years. As a result of these studies, the families of these patients are currently benefiting from accurate carrier-status assessment. Also, some patients carrying a point mutation have been selected for inclusion in clinical trials in view of therapy. However, this technique was limited to a detection rate of 86% in our series. In the aim at identifying point mutation in more patients, we set out to assess the sensitivity of Denaturing High-Performance Liquid Chromatography (DHPLC) and Base Excision Sequence Scanning (BESS) as an approach to mutation screening in DMD. We first assessed a cohort of 15 patients with previously identified mutations (frameshift or stop mutations) spread all over the gene. The comparison of the two methods was based on the analysis of the same amplified fragment of cDNA (~ 500bp) known to contain the mutation. A sequenced cDNA was used as control, and for heteroduplex formation in DHPLC. The sensitivities of DHPLC and BESS were very similar when respectively more than one column temperature was used for the detection of mismatches, and when BESS T and BESS G cleavage reactions were performed. Both allowed the rapid detection of single base substitutions as well as small deletions/insertions. From these findings, we conclude that these two methods provide a reliable alternative to the PTT. Up to now, we have not yet established which of these two technology (BESS or DHPLC) would be the most appropriate for the molecular diagnosis of DMD. We plan to analyze DMD patients (clinical diagnosis confirmed by immunohistochemical analysis of dystrophin) in whom extensive RT-PCR/PTT analysis failed to detect the mutation. Supported in part by a grant of Association Franaise contre les Myopathies (AFM).

**Mutational analysis of BRCA1 in familial breast cancer in Malaysia.** *P. Balraj<sup>1</sup>, A.S.B. Khoo<sup>1</sup>, Y.S. Choy<sup>2</sup>, S.K. Tan<sup>1</sup>, J.A.M.A. Tan<sup>1</sup>, H. Abdullah<sup>2</sup>, C.H. Yip<sup>3</sup>.* 1) Molecular Pathology, Institute of Medical Research, Kuala Lumpur, Malaysia; 2) Kuala Lumpur Hospital; 3) University Hospital, Kuala Lumpur.

Predictive genetic testing of BRCA1 and BRCA2 genes benefits women with family history of breast cancer as it enable medical and lifestyle decisions to be made while reducing the anxiety of not knowing the genetic background. However, the testing for both the BRCA1 and BRCA2 is tedious and expensive as both the genes are large without mutational hot-spots. Mutational testing using direct sequencing detects only 65% of the BRCA-1 linked families. Therefore, uninformative results would limit the benefits of testing in high risk families. In Malaysia, breast cancer is the commonest cancer death among women and it constitutes 7% of all cancers in the country with a prevalence of 150 per 100,000 individuals. Counseling of breast cancer for high risk individuals have been carried out in the breast cancer clinics. A pilot project on BRCA1 mutation analysis was carried out to evaluate the incidence of BRCA1 mutations in individuals at risk and to identify a cost effective methodology. Using direct sequencing of all splice sites and coding sequences, we have identified 2 mutations in two out of 12 Malaysian families (16%) comparable with Japanese families (20%). One of the mutation nt2754 G>A was novel and had been reported (Khoo A.S.B. et al, Human Mutation, 2000). The other mutation nt4693 del AA was found in a large families where 6 living individuals are involved. One frameshift mutation (nt5447 InsC) was detected in 1 out of 23 cases of early-onset breast cancer. Nine polymorphisms were identified and 5 of them were found in exon 11. More than half of the index cases have these polymorphisms. In another blinded study using heteroduplex analysis by conformational sensitive gel electrophoresis (CSGE), all the changes (100%) found by direct sequencing were identified. Therefore, we concluded that CSGE is a useful and equally sensitive method in mutation scanning as direct sequencing. It may be more cost effective to carry out CSGE before sequencing in our setting. But a comprehensive mutation detection strategy should include methodologies which cover large deletions and BRCA2 analysis.

**High throughput and specific newborn screening assay of 21-hydroxylase deficiency in dried blood spots using high performance liquid chromatography/electrospray ionization tandem mass spectrometry.** *C. Lai<sup>1</sup>, C. Tsai<sup>1</sup>, F. Tsai<sup>1</sup>, W. Hwu<sup>2</sup>, J. Wu<sup>1</sup>, S. Chu<sup>3</sup>.* 1) Department of Medical Genetics and Medical Research, China Medical College Hospital, Taichung, Taiwan,; 2) Department of Pediatrics and Medical Genetics, National Taiwan University Hospital, Taipei, Taiwan,; 3) Department of Pediatrics, Buddhist Tzu Chi General Hospital, Hualien, Taiwan,.

A specific, rapid, high throughput, and simple method based on high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) was applied to the screening of congenital adrenal hyperplasia (CAH) caused by 21-hydroxylase deficiency from dried blood spots on newborn screening cards. We used this new method to directly determine the 17OH-P levels in dried blood specimens with a detection limit of 20 ng/ml (~12 ml whole blood was used), to avoid the time-consuming derivatization steps required by gas-chromatography/mass spectrometry (GC/MS) method. The method is reproducible: the CVs were <6%. Dried blood specimens from 500 neonates include neonatal patients with classical 21-hydroxylase deficiency were random analyzed. Ten neonatal specimens had clearly elevated dried blood 17OH-P (>40 ng/ml) at the time that screening for 21-hydroxylase deficiency performed by LC/MS/MS and immunoassay, all were confirmed with true CAH and therefore a cut-off of 40 ng/ml for 17OH-P level was used for CAH newborn screening in our study. This suggested LC/MS/MS were compatible with immunoassay. We aimed at measuring the first dried blood specimens concentrations of 17-hydroxyprogesterone (17OH-P) determined by HPLC/MS/MS in term CAH neonates with 21-hydroxylase deficiency. This LC/MS/MS method is useful for screening infants with CAH due to 21-hydroxylase deficiency.

**VGT-SSCP: A highly sensitive and non radioactive mutation detection method based on vertical gradient temperature SSCP.** *H. Razzaghi, M.I. Kamboh.* Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA.

Single strand conformation polymorphism (SSCP) method is widely used for mutation detection. The sensitivity of the method depends on several factors, most importantly the temperature at which electrophoresis of single-stranded DNA (ssDNA) takes place. Temperature has a profound effect on the folded conformation of ssDNA. The temperature factor is predominantly determined empirically in conventional SSCP, which can be very tedious especially when a large number of different DNA samples need to be screened. We have devised a novel SSCP method based on a vertical gradient temperature (VGT), which automatically subjects ssDNA to various temperatures in the same electrophoresis. The theory behind VGT-SSCP protocol is that when ssDNA is subjected to run in a wide range of gradient temperature, it will automatically acquire optimal resolution at an optimal temperature to distinguish between the wild type and the mutant type ssDNA. The sensitivity level of mutation detection of VGT-SSCP depends on whether the corresponding optimal secondary structure of a mutant DNA strand is within the preset gradient temperature range. In summary, the VGT-SSCP is a simple and robust non-radioactive method that is more sensitive than constant-temperature SSCP in detecting unknown mutations.

**Establishment of stably transformed cell lines from residual clinical blood samples for use in performance evaluation and quality assurance in molecular genetic testing.** *T.T. Stenzel<sup>1</sup>, S.H. Bernacki<sup>1</sup>, J.C. Beck<sup>2</sup>, K. Snow<sup>3</sup>, V.M. Pratt<sup>1</sup>, K.G. Monaghan<sup>4</sup>, T.W. Prior<sup>5</sup>, K.J. Matteson<sup>6</sup>, L.M. Wasserman<sup>7</sup>, W.W. Noll<sup>8</sup>, E.C. Cole<sup>9</sup>.* 1) Duke Univ Medical Center; 2) Coriell Institute for Medical Research; 3) Mayo Clinic; 4) Henry Ford Hospital; 5) Ohio State Univ Hospital; 6) Univ Tennessee Medical Center; 7) Univ California San Diego; 8) Dartmouth-Hitchcock Medical Center; 9) DynCorp Health Research Services Division.

The demand for diagnostic molecular genetic testing (MGT) is continuously increasing as more mutations associated with genetic disorders are characterized. The development of new molecular genetic tests and the performance evaluation and quality assurance (PE/QA) of existing tests require the availability of good positive controls. Currently, these are difficult to obtain for many genetic diseases. We examined the feasibility of using residual blood originally collected for routine clinical MGT to establish stably transformed B lymphocyte cell lines that could supply reliable positive control material. Existing proficiency testing groups use lymphocyte cell lines extensively. In order to obtain the necessary samples, we established a network of 8 sample submitting labs. We used a standard EBV transformation protocol including PHA, and attempted transformation on 45 samples representing 7 genetic diseases. Successful transformation, defined as growth to  $1 \times 10^8$  cells, was achieved in samples up to 14 days old collected in either ACD or EDTA and from as little as 1 ml of residual blood. Tubes were either opened or unopened, and contamination was not a major problem. Acceptable storage conditions were either ambient or 4°C for samples  $\leq 7$  days old and 4°C only for samples 8-14 days old. Average time to transformation was  $43.1 \pm 1.8$  (SEM) days for ACD samples and  $61.3 \pm 10.8$  (SEM) days for EDTA samples. The historical average for samples freshly collected in ACD is  $35.7 \pm 0.3$  (SEM) days. Stability of the mutations was verified after five 10-fold expansions of the cell lines. We plan to pilot the cell lines to outside clinical MGT laboratories to determine their suitability for use as PE/QA material. (Funded by the Centers for Disease Control and Prevention).

**Simplified molecular diagnostic testing for fragile X syndrome using methylation-specific PCR (ms-PCR). Y.**

Zhou<sup>1</sup>, S.S. Chong<sup>1,2,3</sup>. 1) Pediatrics, Nat'l Univ Singapore & Hosp, Singapore; 2) Ob/Gyn & Lab Medicine, Nat'l Univ Singapore & Hosp, Singapore; 3) Pediatrics & Instit Genetic Medicine, Johns Hopkins Univ, Baltimore, USA.

Fragile X syndrome is the most common inherited form of mental retardation and accounts for about half of all X-linked mental retardation. It is caused by mutations in the *FMR1* gene, the overwhelming majority of which involve hyperexpansion and hypermethylation of a polymorphic CGG trinucleotide repeat in the 5' untranslated region of the gene. Currently, molecular analysis of the *FMR1* gene CGG repeat is performed using a combination of PCR and Southern blot analyses, neither of which can be used exclusive of the other to detect the full spectrum of *FMR1* alleles and genotypes. We explored the use of sodium bisulfite mediated selective modification of unmethylated versus methylated genomic DNA at the *FMR1* locus to develop a ms-PCR diagnostic testing strategy. Primers specific to sodium bisulfite modified unmethylated and methylated alleles were designed and tested on DNA samples from normal, premutation and full mutation males, as well as from normal, premutation carrier and full mutation affected females. For each sample, the sizes of the ms-PCR amplicons obtained using the unmethylated-specific and methylated-specific primers were compared to the corresponding results obtained by Southern blot analysis of *EcoRI/EagI* double-digested DNA. For most samples tested, ms-PCR amplicons of expected sizes from methylated and unmethylated alleles were clearly detectable by ethidium bromide staining on agarose gels. The assay also faithfully reflected methylation status of the respective alleles each sample, as confirmed by Southern blot analysis. Premutation and full mutation alleles in females, however, were consistently weaker in intensity than their corresponding normal allele, reflecting a lower amplification efficiency compared to the normal allele. Further modifications to improve detection of large premutation (> 100 repeats) and full mutation alleles are in progress, including the incorporation of fluorophore-tagged dNTPs during PCR, followed by automated fluorescence detection.

Program Nr: 1498 from the 2001 ASHG Annual Meeting

**A comprehensive molecular analysis of myotonic dystrophy in Iran.** *M.S.M.E. Mousavi, K. Kahrizi, S.S Hosseini Amini, H. Najmabadi.* Genetics Research center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.

Myotonic dystrophy (DM) is the most common form of adult muscle dystrophies with varying frequencies in different populations. So far there has been no comprehensive population screening on this disease in Iran. DM has multisystemic manifestation including muscle weakness and myotonia. It has pattern of Trinucleotide (CTG) repeats, in 3' untranslated region of the serine Threonine protein kinase gene located on chromosome 19q13.3. Due to similarity in clinical picture with the other dystrophic patients and the lack of reliable diagnostics we decided to set up a molecular analysis technique for DM for the first time in Iran. PCR and southern blotting were performed using nonradioactive material. 100 patients were investigated. DM gene mutations were detected in 35% of the clinically diagnosed DM patients.



**Quality evaluation of data interpretation and reporting.** *E. Dequeker, J.-J. Cassiman.* Center for Human Genetics, KU Leuven, Leuven, Belgium.

Errors in any of the steps of a molecular genetic test may affect its results and conclusions, and can have potential consequences for prenatal diagnosis and carrier testing. Patients are usually tested only once and an incorrect result may not become apparent for many years. It is important that the error rate of genetic tests should be reduced to an absolute minimum. Over the past five years quality assessment schemes for cystic fibrosis (CF) were organised in Europe where 135 to 200 diagnostic laboratories participated. A significant improvement of the quality of the genotype results was obtained during subsequent QA schemes, coming from 65% of laboratories without errors in 1996 up to 90% in 2000. In addition to the evaluation of the genotype results, evaluation of the interpretation of the data in the report that is normally sent to the referral physician was done since 1999. The schemes demonstrated that the way of reporting laboratory results varied considerably, although the same request for genetic testing was addressed to all participants. A list of relevant items in the written reports was prepared. Based on the presence of the items of this list in the reports of 1999 versus 2000 we conclude that the standard of reports has improved in 2000. In particular, sample and patient information has improved, together with technical information. Unfortunately, more than 30% of the laboratories made at least one mistake in a submitted report (administrative errors, errors in risk calculation, a wrong interpretation of the results, or confusing genotype results of samples from patients and carriers). Taking into account the different reporting policies (country specific issues), this evaluation study also provided information on the large variation between laboratories in reporting genetic testing results for carrier testing of individuals with a positive family history of CF, or for genetic confirmation testing of the clinical diagnosis of CF. Overall, there is a high variation in the format, the content, and the quality of the written reports on molecular genetic tests. This could be further improved by preparing and using consensus guidelines for genetic test reporting (including the availability of model laboratory reports).

**A New Approach To Detect Gene Dosage At The *PMP22* Gene.** *S.M. Akrami, J.A.L. Armour.* Institute of Genetics, University of Nottingham , Nottingham, UK.

An increasing number of human disorders are known to be caused by micro-deletions/duplications, and gene dosage alterations are responsible for several categories of diseases. Examination of sub-microscopic changes is possible by using short probes flanked by the same primer pairs and such probes can be recovered and amplified quantitatively following hybridization to a genomic target. Multiplex Amplifiable Probe Hybridization (MAPH) is a simple, high-resolution method by which alterations of several hundred base pairs are detectable. DNA rearrangement is the molecular cause of most Charcot-Marie-Tooth (CMT1) disease by a gene dosage mechanism: the 1.5Mb duplication of chromosome 17p11.2-12 containing the *PMP22* gene accounts for about 70% of CMT1 patients. A deletion of the same region is detected in more than 85% of cases of hereditary neuropathy with liability to pressure palsies (HNPP). We are applying MAPH on the *PMP22* gene to develop an efficient and sensitive test for detecting gene dosage in CMT1A/HNPP patients. Work on genomic DNA of normal people shows reproducible results approximating to a normal distribution. A preliminary blind test of 99 samples gave the correct diagnosis in all but two cases; the method is currently being refined to investigate and eliminate cause of these two false-negative calls.

**Rapid detection of b-thalassemia mutations in the Italian population using a DHPLC system.** A. Colosimo<sup>1,2</sup>, V. Guida<sup>1,3</sup>, A. De Luca<sup>1</sup>, M.P. Cappabianca<sup>4</sup>, I. Bianco<sup>4</sup>, B. Dallapiccola<sup>1,3</sup>. 1) CSS-Mendel Institute, Rome, Italy; 2) Department of Biomedical Sciences, University "G.D'Annunzio" of Chieti, Italy; 3) Department of Experimental Medicine and Pathology, University "La Sapienza" of Rome, Italy; 4) Associazione nazionale per la lotta contro le Microcitemie in Italia.

b-thalassemia is the most common mendelian disease in the Mediterranean area, resulting from more than 200 different mutations in the b-globin gene. Prevention programs based on postnatal and prenatal molecular diagnosis of b-thalassemia carriers and patients require the use of a reliable mutation scanning method in at-risk populations. We have developed a rapid and highly specific mutation screening test based on the Denaturing High Performance Liquid Chromatography (DHPLC) system. The sensitivity and specificity of the method were tested on the full genomic region of the b-globin gene in 40 Italian healthy heterozygous carriers in which 25 different b-globin mutations had been previously characterized by ARMS-PCR technique. The entire gene, including 280 bp upstream of exon 1 and 200 bp downstream of exon 3, was amplified in 7 fragments of 300-500 bp using identical PCR parameters. The sensitivity and specificity reached 100% since all the 25 distinct sequence alterations were precisely identified by DHPLC. There were neither false positive nor false negative results. Both missense mutations and single or double base deletions were detected. In addition, several compound heterozygous and homozygous b-thalassemia patients were successfully subjected to DHPLC. To detect the mutations in homozygous patients the DHPLC analysis was preceded by a mixing step with a reference wild-type DNA. Overall, the method was able to unambiguously identify the most common b-thalassemia mutations, accounting for more than 95% of b-globin alleles in the Italian population. However, the presence of possible polymorphisms recommends to perform sequencing on DNA samples, whose DHPLC chromatograms differ from characterized elution profiles. In conclusion, compared to classical approaches of mutation screening, this method allows a rapid, highly sensitive, cost effective and semi-automated mutational screening of a large number of samples.

**Development and evaluation of a multiplexed, automated assay using a commercially available primer extension kit for determining the African American 3120+1G>A and the French Canadian I148T mutations in Cystic**

**Fibrosis.** *N.M. Brown<sup>1</sup>, S. Bernacki<sup>1</sup>, V.M. Pratt<sup>2</sup>, T.T. Stenzel<sup>1</sup>.* 1) Dept of Pathology, Duke University, Durham, NC; 2) Laboratory Corporation of America, Research Triangle Park, NC.

Cystic Fibrosis (CF) is an autosomal recessive disorder that is characterized by the triad of chronic broncho-pulmonary disease, pancreatic insufficiency and elevated sweat electrolytes (Welsh et al., 1995). Over 900 mutations in the CFTR gene have been found to cause CF and congenital bilateral aplasia of the vas deferens. The American College of Medical Genetics (ACMG) has recommended a core mutation panel for general population CF carrier screening (Grody et al., 2001). This panel contains 25 of the known CF mutations including 3120+1G>A and I148T. Commercially available kits (Roche, Applied Biosystems and Innogenetics) contain most of these 25 mutations but usually do not include 3120+1G>A and I148T. Therefore we developed a multiplex primer extension assay to rapidly detect these two CF mutations using the ABI prism Snapshot ddNTP primer extension kit. The kit uses a single fluorescently labeled dideoxynucleotide primer extension reaction in conjunction with the ABI genetic analyzer to detect the addition of a fluorescently labeled ddNTP to the 3' end of a sequencing primer, identifying the specific polymorphism or mutation. Seven samples were used to verify our assay's ability to identify normal and mutated alleles. From each sample, PCR amplified regions that included the mutations (3120+1G>A and I148T) were multiplexed into a single dideoxynucleotide primer extension reaction using two different sequencing primers of different lengths. Analysis was performed on the ABI 310 genetic analyzer, and five normal samples and two heterozygotes samples were identified. Multiplexing the single-plex ABI Prism SNaPshot ddNTP primer extension kit allows rapid identification of normal specimens and mutated specimens that carry the CF 3120+1G>A and I148T mutations. The successful development of this multiplex assay has enabled us to speculate that further manipulation of the assay could allow more than two mutations to be analyzed by using the single-plex kit resulting in a cost saving.

**Validated Standards for Genetic Testing.** *J.C. Beck, C.M. Beiswanger, L.H. Toji, P.K. Bender, J.C. Leonard, R.T. Johnson.* Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ.

As the number of molecular genetic diagnostic tests offered continues to increase, there is a critical need for validated standards accessible to laboratories offering these tests. The Coriell Cell Repositories through the NIGMS Human Genetic Cell Repository and the NIA Aging Cell Repository have a collection of more than 600 cell lines and DNA samples representing 83 diseases with characterized mutations which could be used as standards. These include diseases caused by expansion of trinucleotide repeats, such as dentatorubral-pallidoluysian atrophy (for which three samples with known repeats are available); myotonic dystrophy (13); Friedreich ataxia (10); fragile X syndrome (26); Huntington Disease (13); SCA1 (2); and SCA3 (2). The collection also includes 40 different mutations in the *CFTR* gene, 20 unique mutations in the *BRCA1* gene, 6 mutations in the *BRCA2* gene, and 4 mutations in the *APC* gene. Samples from patients with hemochromatosis (19), muscular dystrophy (11), and spinal muscular atrophy (3) have also been molecularly characterized. In addition, specimens carrying the factor V Leiden mutation (4), the *MTHFR* thermolabile variant (3), and the 20210G-A polymorphism in the prothrombin gene (2) are also included in the collection. Standards are also available for apolipoprotein E and Rh D genotyping. Finally, the collections include five cell lines with mutations in multiple genes: two have mutations in three different genes, e.g., one with mutations in *MTHFR*, *F2*, and *F5*, and a second with mutations in *MTHFR*, *F2*, and *HFE*, and three cell lines have identified mutations in two genes, e.g., *MTHFR* and *DMPK*, *HFE* and *F5*, and *CFTR* and *HFE*. The samples in these resources, validated by certified expert molecular laboratories, are valuable reagents for laboratories performing molecular genetic testing and may also be useful for quality assurance programs. Detailed information about these samples, including ordering instructions, is available in an electronic catalog (<http://locus.umdj.edu/ccr>).

**Molecular Diagnosis of Five Spinocerebellar Ataxias by Capillary Electrophoresis.** *M.O. Dorschner<sup>1</sup>, D. Barden<sup>2</sup>, K. Stephens<sup>1,2</sup>*. 1) Division of Medical Genetics; 2) Department of Laboratory Medicine, University of Washington School of Medicine, Seattle, WA.

We have developed a single-tube, multiplex assay for the analysis of the CAG repeat expansions that cause the five most prevalent spinocerebellar ataxias, SCA1, 2, 3, 6, and 7. To facilitate multiplex amplification, an M13 -21 universal sequence was added to the 5' end of each SCA-specific primer, which is thought to mitigate differences in annealing and amplification efficiencies. By judiciously pairing fluorescent dyes with each SCA locus such that products of overlapping size ranges were labeled differently, only the three fluorescent dyes FAM, HEX, and NED were needed. All five loci amplified simultaneously using a *Pwo/Taq* polymerase blend. Both normal and expanded alleles were detected reproducibly, although expanded alleles amplified less efficiently as expected. The interassay variability is less than a single base. To assess the accuracy of fragment sizing by capillary electrophoresis (CE), we assayed samples that had been previously analyzed by conventional polyacrylamide gel electrophoresis (PAGE). Initial fragment sizes using CE were determined relative to a commercially available size standard. CE underestimated the fragment lengths for all five loci. This phenomenon is presumably caused by the secondary structure of amplicons and has been described previously for the trinucleotide repeat expansions of the Huntington and fragile X genes. Regression analysis of fragment length determined by PAGE versus CE was employed to calculate a size correction factor for each SCA locus. Size correction factors differed significantly among the five loci. The dominant spinocerebellar ataxias are a group of progressive neurodegenerative disorders that cannot be diagnosed accurately in the clinical setting. This assay will facilitate rapid, specific, and precise molecular diagnosis.

**The prevalence of mutations that cause Familial Dysautonomia and Mucopolysaccharidosis Type IV in the Ashkenazi Jewish Community.** *L. Edelmann, J. Dong, R.J. Desnick, R. Kornreich.* Dept Human Gen, Box 1497, Mt Sinai Sch Medicine, New York, NY.

Carrier screening among the Ashkenazi Jewish (AJ) population for a number of autosomal recessive mutations has provided an extremely effective method for identifying individuals at risk for having children with severely debilitating and often lethal diseases. For the majority of these diseases, there is currently no cure. Recently, the genetic lesions responsible for two additional recessive disorders that affect primarily AJ individuals were identified. They include, two mutations in the I $\kappa$ B kinase complex associated protein gene (*IKBKAP*), which are responsible for Familial Dysautonomia (FD), a severe congenital neuropathy, and two mutations in *MCOLN1*, which cause Mucopolysaccharidosis Type IV (MLIV), a severely debilitating lysosomal storage disease. To determine the frequency of carriers for both of these disorders and discern whether they should be added to the AJ panel, we anonymously tested DNA samples with informed consent from AJ individuals, living in the greater New York Metropolitan area, that were screened for other disorders in our current AJ panel. The use of a multiplex PCR method coupled with allele specific oligonucleotide hybridization (ASOH) facilitated a simultaneous search for mutation carriers for both disorders. For FD, 2500 AJ individuals were analyzed for the presence of the IVS20<sup>+6T>C</sup> splicing major mutation and the R696P missense minor mutation in *IKBKAP*. A carrier frequency of 3.2% or 1 in 31 was found for the splicing mutation, but we did not identify any carriers of the missense mutation, confirming that the IVS20<sup>+6T>C</sup> major mutation accounts for nearly all cases of FD in the AJ population. For MLIV, 1100 AJ individuals were screened for two founder mutations in *MCOLN1*, the major 5534A>G splicing mutation and the minor 511>6944del, a 6434 bp deletion. For the splicing mutation, a carrier frequency of 0.45% was found, and for the deletion, the frequency of carriers was 0.18%. These mutations have a combined frequency of 0.64% indicating that 1 in 157 AJ individuals is a carrier for MLIV. Based on these findings, we have added FD to our AJ screening panel and are in the process of including MLIV screening as well.

**Dutch approach to confirm the clinical diagnosis CADASIL, five new Notch3 gene mutations.** *E. Bakker<sup>1</sup>, S.A.J. Lesnik Oberstein<sup>1</sup>, M.J. Vollebregt<sup>1</sup>, J. Haan<sup>2</sup>, M.D. Ferrari<sup>2</sup>, M.H. Breuning<sup>1</sup>.* 1) Dept of Human and Clinical Genetics,; 2) Dept of Neurology, LUMC, Leiden, Netherlands.

The Dutch CADASIL Research Group uses a stepwise approach to confirm, molecular genetically, the clinical diagnosis in individual patients with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Based on our earlier results, detecting a mutation in 11 out of 11 clinically well described CADASIL patients by sequence analysis of the Notch3 gene (1), we developed a stepwise approach making use of the Notch3 gene mutation spectrum (2), clinical and MRI data. For clinically suspected cases we start with sequence analyses of exon 4 (sequence round 1), which harbours a mutation hotspot (~65%; of all known mutations). When no mutation is detected in round 1 the referring physician is requested to send clinical and MRI data. If these proofs to support the diagnosis CADASIL, further analysis of the Notch3 gene (the remaining 32 exons) is performed. This proof could be detailed MRI data, because all genetically proven patients sofar showed prominent signal abnormalities on brain MRI -leukoencephalopathy on T2- and small subcortical infarcts on T1-weighted images. Or a positive pedigree, with several cases of late onset recurrent TIAs and strokes, vascular dementia, migraine with aura (35%; of patients), and psychiatric disturbances (30%; of patients). In those clinically highly suspected cases further analysis of the Notch3 gene is conducted in several rounds of sequencing. In round 2, exons 3, 5, 11, and 19 are sequenced. In round 3, the exons 2, 8, 14, 18, 20, 22 and 23 are sequenced. The last round harbours 16 of the remaining 18 exons, sequencing of exon 1 and 33 is not yet operational. Up to now 15 different mutations were detected, some of which recurrent and at least 5 which to our knowledge were not yet described: Cys134Trp, Cys446Phe, Arg544Cys, Cys1015Arg and Arg1076Cys. 1) SAJ Oberstein et al. (1999) Neurology 52;1913 2) A Joutel et al. (1997) The Lancet 350;1511-1515.



**Use of Invader assays to determine allele frequency of SNPs involved in lipid metabolism.** *B. Aizenstein, M. Baker, M. Brink, M. Maguire, R. Grygiel, K. Osterby, B. Neri, M. de Arruda.* Third Wave Technologies, Madison, WI.

The regulation of plasma lipid levels is the result of the complex interaction of the many gene products involved in their transport and metabolism. Genetic variations in these genes have been associated with a variety of diseases including hypercholesterolemia, hyperlipoproteinemia, and hypertriglyceridemia. The number of genes involved in lipid metabolism and the large number of variations that have been described in each gene, make their characterisation particularly important in accurately identifying the genetic basis of these diseases. We have used the Invader Assay to characterise over 20 allelic variations contained within 5 different genes involved in circulating plasma lipid metabolism: Low-Density Lipoprotein Receptor (LDLR), Lipoprotein Lipase (LPL), Cholesterol Ester Transfer Protein (CETP), ATP-Binding Cassette (A1 and C4 subfamilies), and Apolipoprotein E (ApoE).

The Invader® Assay is a signal amplification method for rapid and inexpensive identification of genetic variations directly from genomic DNA samples. Signal generation is based on the presence of an invasive specific structure formed by the hybridisation of two oligonucleotides, Primary Probe and Invader oligo, to the target DNA. The Primary Probe includes a 5'-flap that does not hybridize to the target DNA. This invasive structure is the substrate for the Cleavase enzyme, which in turn cleaves off the unpaired 5'-flap of the primary probe. The length of sequence specific region of the probe is designed to cycle on and off the target sequence at the reaction temperature thus allowing multiple cleaved flaps to be generated from a single target molecule. In a secondary concomitant reaction, the released flap serves as an Invader oligo on a synthetic target-probe complex, termed FRET Cassette, to recreate the invasive structure recognized by the Cleavase enzyme. Signal generation occurs when the Cleavase enzyme recognises and cleaves the invaded FRET cassette releasing the fluorescent dye.

We will present data on the allele frequency of each of the studied genes using a panel of 92 individuals.

**Comparison of methods for detection of mutations in the BRCA1 gene.** *B.A. Bove<sup>1</sup>, H. Ozcelik<sup>2</sup>, S. Neuhausen<sup>6</sup>, J. Boyd<sup>3</sup>, M. Southey<sup>9</sup>, R. Santella<sup>7</sup>, D. Venter<sup>9</sup>, J. Beck<sup>1</sup>, F. Li<sup>5</sup>, S. Buys<sup>4</sup>, I.L. Andrulis<sup>2</sup>, A.K. Godwin<sup>1</sup>, A. Whittemore<sup>8</sup> and CFRBCS.* 1) Fox Chase Cancer Ctr, Philadelphia, PA; 2) University of Toronto; 3) Memorial Sloan Kettering Cancer Center; 4) Huntsman Center; 5) Dana Farber Cancer Center; 6) University of Utah; 7) Columbia University; 8) Stanford University; 9) Peter MacCallum Cancer Institute.

The Cooperative Family Registry for Breast Cancer Studies (CFRBCS) was established in 1995 to create a resource that could be used to investigate genetic and environmental factors that influence the susceptibility to breast cancer and to exploit this information to aid in preventing the disease. Families in the CFRBCS have been identified through population-based registries in Northern California, Ontario and Australia and high-risk clinics in Pennsylvania, New York, Utah and Australia. Knowledge of the germline BRCA1 and BRCA2 status of individuals in the CFRBCS is an essential first step in utilizing the Registry for molecular epidemiological studies of genotype-phenotype correlations, gene-gene and gene-environment interactions. The purpose of the present study was to determine the sensitivity and specificity of molecular techniques to detect mutations in the BRCA1 gene. Twenty one coded lymphoblastoid cell line samples, whose BRCA1 mutation status had been determined previously by direct DNA sequencing analysis, were evaluated for DNA alterations using the following mutation detection assays: single-strand conformation polymorphism analysis (SSCP), two dimension (2-D) gene scanning, protein truncation test, and enzyme mutation detection (EMD). PTT correctly identified all truncation mutations. The EMD method correctly identified all truncation mutations, with the expected exception of a large deletion in exon 22, as well as two missense alterations. Two mutations were not detected by 2D gel scanning, and seven deleterious mutations were not detected by SSCP. A combination of techniques identified one additional truncation and one additional unclassified variant than was detected by direct sequencing, suggesting that complimentary methods for mutational analysis may be necessary.

**The relative contribution of mutations in the DFNB loci to congenital/early childhood non-syndromal sensorineural hearing impairment/deafness.** *N.C. Navarro-Coy<sup>1</sup>, T.P. Hutchin<sup>1</sup>, H.E. Conlon<sup>2</sup>, E.L. Coghill<sup>3</sup>, A. Middleton<sup>4</sup>, J.S. Rowland<sup>4</sup>, G.R. Taylor<sup>4</sup>, T. Bishop<sup>5</sup>, R.C. Trembath<sup>2</sup>, S.D.M. Brown<sup>3</sup>, R.F. Mueller<sup>1,4</sup>.* 1) Molecular Medicine Unit, University of Leeds, Leeds, UK; 2) Division of Medical Genetics, University of Leicester, UK; 3) MRC Mammalian Genetics Unit, Harwell, UK; 4) 4 Clinical Genetics, St James's University Hospital, Leeds, UK; 5) 5 ICRF Genetic Epidemiology Unit, St James's University Hospital, Leeds, UK.

Approximately 1 in 1000 children have congenital/early childhood onset sensorineural hearing impairment, of which 50% are genetic, two-thirds of which are non-syndromal with some three-quarters thought to be due to genes inherited in an autosomal recessive manner. Mutations in connexin 26 (CX26) have been identified as the most common cause, establishing CX26 gene mutation testing as a routine diagnostic investigation for a child sporadically affected with non-syndromal sensorineural hearing impairment/deafness. The relative contribution of the remaining recessive loci (DFNB) to autosomal recessive non-syndromal sensorineural hearing impairment/deafness (ARNSSNHI/D) is unknown. We analysed the contribution of the DFNB loci in a cohort of 118 sib pairs with ARNSSNHI/D. Cx26 mutations were found to be responsible in 28/118 (23.7%) of the sib-pairs. Analysis of the flanking linked polymorphic microsatellite markers for each of the remaining DFNB loci were analysed in the remaining sib pairs for concordance at each locus. All of the remaining loci were concordant for a minority of the sib-pairs, suggesting that none of the genes at the other DFNB loci appear to make a major contribution to ARNSSNHI/D. However, initial results of screening the DFNB genes which have been cloned revealed mutations in the PDS gene in 3 of the 10 sib-pairs concordant for the DFNB4 locus. The results of this study provides preliminary evidence for a rational clinical and molecular investigation of children sporadically affected with congenital/early childhood onset non-syndromal sensorineural hearing impairment.

**Denaturing high-performance liquid chromatography reliably detects ion channel mutations in Long QT syndrome.** *L. Ning<sup>1,2,3</sup>, A. Moss<sup>3</sup>, W. Zareba<sup>3</sup>, J. Robinson<sup>3</sup>, S. Rosero<sup>3</sup>, D. Ryan<sup>1</sup>, M. Qi<sup>1,2,3</sup>.* 1) Dept. of Pathology & Laboratory Medicine, University of Rochester, Rochester, NY; 2) Center for Cardiovascular Research, University of Rochester, Rochester, NY; 3) Dept. of Medicine, University of Rochester, Rochester, NY.

The hereditary long QT syndrome (LQTS) is a familial cardiac disorder that causes syncope, seizures, and sudden death from ventricular arrhythmias, specifically torsade de pointes. Multiple heterozygous mutations in several ion channel genes (KvLQT1, HERG, SCN5A, KCNE1, and KCNE2) have been shown to cause autosomal dominant LQTS. Due to their multiple loci and considerable sizes (2.8 kb of the coding sequence, represented by 16 exons for KvLQT1; 4 kb of the coding sequence, represented by 15 exons for HERG; 6.2 kb of the coding sequence, represented by 28 exons for SCN5A), mutation detection in these genes represents a challenge that is only partially met by the conventional screening method of single stranded conformational polymorphism (SSCP). The current technique is time consuming and labor intensive, requires specific expertise and is limited to a detection rate of 90%. In contrast, the recently introduced denaturing high performance liquid chromatography (dHPLC) offers a promising new method for a fast and sensitive analysis of PCR-amplified DNA fragments. To test the applicability of dHPLC in the molecular diagnosis of LQTS, we first assessed a cohort of 172 patients from our International LQTS Registry with 12 previously identified by SSCP and/or sequencing mutations (including 8 different missense mutations, 1-bp, 2-bp, 3-bp and 9-bp deletion mutations), and 2 polymorphisms in the LQTS genes. Eighty-two patients carry one or more mutation/polymorphism (s), and the remaining 90 patients are negative controls. Applying empirically determined exon-specific melting profiles, all mutations and polymorphisms were detected readily and reproducibly. One mutation undetectable by SSCP previously was also identified by dHPLC. From these findings we conclude that the dHPLC technology is a highly sensitive and efficient method for the molecular analysis of LQTS.

**Evaluation of DHPLC as a screening method for mutation detection in molecular diagnostics.** *L. Han<sup>1</sup>, A. Sekowski<sup>1</sup>, C. Wei<sup>1</sup>, Y. Qi<sup>1</sup>, T.L. Stockley<sup>1,2</sup>, P.N. Ray<sup>1,3</sup>.* 1) Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada; 3) Department of Molecular and Medical Genetics, University of Toronto, Ontario, Canada.

The demand for efficient direct mutation detection in large genes for molecular diagnostics is rapidly increasing. Improved screening techniques to highlight regions of a gene that potentially contain mutations will be required to meet this demand. Denaturing high-pressure liquid chromatography (DHPLC) is a sensitive, automated screening method for heterozygote detection. In DHPLC DNA fragments are separated by HPLC at a temperature that results in partial denaturation of duplex DNA to distinguish heteroduplexes containing mutations from normal homoduplexes. The objective of this study is to evaluate DHPLC as a prescreening method prior to direct sequencing for molecular diagnostics, using analysis of Fabry disease, Retinoblastoma and Hunter syndrome as examples. Exons of the GLA gene (Fabry), RB-1 gene (Retinoblastoma) and IDS gene (Hunter) from patients and carriers with known mutations were analyzed with DHPLC at temperatures and buffer gradient conditions necessary for partial denaturation as predicted by WAVEMAKER® software (Transgenomics). Resulting chromatograms were analyzed and variants compared to normal control samples. Any exons that showed variants in DHPLC were sequenced in both directions to detect any sequence changes. 7 exons of GLA, 9 exons of IDS and 28 exons of RB-1 in 58 patient samples were screened by DHPLC for a total of 481 exon fragments analyzed. Of 74 fragments with known sequence changes, 72 were detected by DHPLC to give a sensitivity of 97%. 2 fragments with known sequence changes were not detected by DHPLC, to give a false negative rate of 2.7%. 23 of 407 fragments that did not have sequence changes were positive on DHPLC, to give a false positive rate of 5.6%. Therefore DHPLC has a high sensitivity and low false negative and false positive rates as indicated by the analysis of the GLA, IDS and RB-1 genes and is useful in expediting molecular diagnosis of these disorders.

**Complete Analysis of the PKD1 and PKD2 Genes Responsible for Polycystic Kidney Disease: a Clinical Testing Service.** *J.G. Jones<sup>1</sup>, J.A. Garces<sup>1</sup>, J. Wang<sup>1</sup>, J. Curran<sup>1</sup>, S. Allen<sup>1</sup>, K. Flynn<sup>1</sup>, A.N. Hennigan<sup>1</sup>, C.M. Sullivan<sup>1</sup>, N. Robichaud<sup>1</sup>, G.G. Germino<sup>2</sup>, T.J. Watnick<sup>2</sup>, S. Somlo<sup>3</sup>, W.K. Seltzer<sup>1</sup>, S. Hallam<sup>1</sup>, L. Kertiles<sup>1</sup>, C.M. Palatucci<sup>1</sup>, M.A. Boss<sup>1</sup>.* 1) Athena Diagnostics, Inc., Worcester, MA; 2) Johns Hopkins University School of Medicine, Baltimore, MD; 3) Yale School of Medicine, New Haven, CT.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the leading causes of kidney failure. Mutations scattered throughout two genes, PKD1 and PKD2, cause about 95% of all ADPKD. Development of a DNA-based diagnostic tool for routine clinical use has been hindered by 3 factors: (1) 70% of the PKD1 gene is replicated as non-functional homologues with >95% sequence identity to PKD1, (2) portions of the gene are extremely GC rich, and (3) 15816 base pairs need to be analyzed. We developed a DNA testing service that comprehensively screens the coding sequence of both PKD genes to assist in the diagnosis and management of ADPKD. All 15816 bp of coding sequence as well as the invariant splice sites are evaluated. Genomic DNA extracted from whole blood is used for 8 highly specific long-range PCRs spanning the PKD1 replicated region. Primers for these LR PCRs are carefully designed to be PKD1-specific, being positioned at rare sites where the PKD1 sequence differs from the homologues. The 8 long range PCR products are diluted at least 1000 fold to minimize homologue sequence present in the residual genomic DNA, and are then used to amplify 52 nested fragments spanning the 34 PKD1 exons replicated in the homologues. These crucial steps prevent the spurious amplification of PKD1 homologues that would otherwise confound the analysis. Genomic DNA is used directly for 31 additional PCRs spanning the unique region of PKD1 and the entire PKD2 gene. All 83 PCR products are analyzed by denaturing HPLC (DHPLC) and those with variants are sequenced to identify the DNA alteration. Approximately 80 different PKD-specific variants were developed as positive controls for DHPLC analysis. Analytical sensitivity is derived from the analysis of over 120 different DNA variants scattered in all regions of the genes.

**Automated simultaneous analysis of 22 beta-globin mutations by reverse-hybridization.** *C. Oberkanins, A. Moritz, F. Kury.* ViennaLab Labordiagnostika GmbH, A-1110 Vienna, Austria.

More than 150 mutations in the human beta-globin gene have been identified to date, either causing structural abnormalities, such as hemoglobin S (sickle cell anemia), or leading to synthesis disfunctions (beta-thalasseмии). The resulting disorders are a major public health problem, in particular among Mediterraneans, Asians and Africans. Since for the large majority of affected individuals there is only supportive management but no definitive cure, emphasis is given to prevention programs based on heterozygous carrier screening and prenatal diagnosis. We have developed a reverse-hybridization assay (Beta-Globin StripAssay) for the rapid and simultaneous detection of 22 common beta-globin mutations. The test is based on a single, multiplex DNA amplification reaction and ready-to-use test strips containing oligonucleotide probes for each wild-type and mutated allele immobilized as parallel lines. It allows to discriminate between heterozygous ("minor"), compound heterozygous and homozygous ("major") individuals from one single test strip. The coverage with respect to the mutation spectrum is population-specific, reaching 80-99% in Mediterranean and Middle Eastern countries. The entire procedure from blood sampling to the identification of beta-globin mutations requires less than 6 hours, and may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot).

**Alpha thalassemia In Iran.** *M. Neishabury*<sup>1</sup>, *L. Abbasi Moheb*<sup>1</sup>, *A.A. Poorfathollah*<sup>2</sup>, *K. Kahrizi*<sup>1</sup>, *E. Keyhany*<sup>1</sup>, *W. Krugluger*<sup>3</sup>, *Ch. Oberkanins*<sup>4</sup>, *H. Najmabadi*<sup>1</sup>. 1) Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran; 2) Blood Transfusion Services, Tehran, Iran; 3) Institute of Clinical Chemistry, Rodlfstiftung Hospital, Vienna, Austria; 4) Vienna lab, Labordiagnostka, Vienna, Austria.

The most common mutations of alpha thalassemia are the deletion mutations including single or double gene deletions. -a3.7 and -a4.2 are the most common single gene deletions. -a3.7 has a world wide distribution among all racial groups and -a4.2 is less common being most prevalent in South East Asia and Saudi Arabia. In Iran, a large number of patients with reduced MCV and MCH, normal HbA2 and F levels and normal iron level are being referred to molecular biology clinics all over the country as suspicious cases of alpha thalassemia. This causes a concern to determine the common alpha thalassemia mutations for Iranian population. This will greatly help us to do carrier identification and prenatal diagnosis for prevention of the deleterious forms of alpha thalassemia such as Hb H and Hb Barths's. In addition in a country like Iran with a considerably high prevalence of beta thalassemia, co inheritance of alpha and beta thalassemia is expected to occur in high frequency. In this study, the frequency of the two most common alpha thalassemia mutations in the region, -a3.7 and -a4.2 were determined in 60 alpha thalassemia suspicious cases. 33 % of the alpha thalassemia mutations in the studied population was -a3.7 while no -a4.2 deletion was identified.



Program Nr: 1515 from the 2001 ASHG Annual Meeting

**An automated multiplex detection system for detection of haemoglobinopathy mutations.** *Y.T. Liu, D.J. Weatherall, J.B. Clegg.* MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, U.K.

The inherited disorders of haemoglobin - haemoglobinopathies are some of the most common single-gene disorders in humans. The World Health Organization has estimated that approximately 7 per cent of the world's population are carriers for these disorders, which include sickle cell anaemia and the thalassaemias.

Although common haemoglobinopathy mutations can now be routinely detected by PCR-based methods, like gap PCR, ARMS and reverse dot blotting, none of these methods easily lend themselves to an automated multiplexing approach. A method, which is based on the oligonucleotide ligation assay (OLA), that allows multiplex detection of non-deletion and deletional  $\alpha$  thalassaemia variants as well as  $\beta$  thalassaemia point mutations in a single reaction, will be presented. The method uses currently available automated DNA sequencing instrumentation and software. We envisage that it can form the basis of a fully comprehensive automated system which will be capable of detecting more than 99% of the  $\alpha$  and  $\beta$  globin gene mutations that underlie the major haemoglobinopathies.

**High-Through-Put SNP Detection and Haplotype Assessment in Human Melanoma Inhibitory Activity like Gene**

**(MIAL).** *P. Mouritzen<sup>1</sup>, A.T. Nielsen<sup>1</sup>, Y. Choleva<sup>1</sup>, L. Kongsbak<sup>1</sup>, N.D. Rendtorff<sup>2</sup>, N. Tommerup<sup>2</sup>, H. Vissing<sup>1</sup>.* 1) Euray, Exiqon A/S, Bygstubben 9, 2950 Vedbaek, Denmark; 2) Dept. of Medical Genetics, Inst. of Medical Biochemistry and Genetics, the Panum Inst., University of Copenhagen, 2200 Copenhagen N, Denmark.

The Human Melanoma Inhibitory Activity like Gene (MIAL) encodes a small extracellular protein that is preferentially expressed in the inner ear in humans. A frequent polymorphism exists in the translation initiation codon of the MIAL gene (+2 SNP), which abolishes the translation of the gene into protein. The restricted expression pattern and the inactivating polymorphism suggest that MIAL may contribute to inner-ear dysfunction in humans.

We have developed a high-through-put SNP detection technology, which potentially may genotype more than 35.000 patients for two SNPs in a single microarray experiment. Results will be presented using the +2 SNP and another upstream SNP (-31 SNP) of the MIAL gene as an example. MIAL amplicons containing the two SNPs are produced from each patient DNA sample with high fidelity PCR using LNA (Locked Nucleic Acid) containing primers. The amplicons are spotted on plastic slides followed by hybridization with four LNA containing probes each labeled with a different fluorophore. The LNA oligonucleotide probes complement the four possible sequences containing the two SNPs. A further development of the techniques allow us to perform haplotyping on chromosomal DNA. With the two MIAL SNPs we demonstrate haplotyping using a combination of allele specific PCR and hybridization of the resulting amplicons to arrayed capture probes on slides. Again the fidelity of the system relies on LNA in the allele specific primers and in the capture probes. The allele specific primers discriminate between the -31 SNP genotypes and capture probes discriminate between the +2 SNP genotypes. Hybridization of the different allele PCR amplicons are distinguished from each other by labeling the two allele specific primers with different fluorophores. The developed SNP detection technologies provide a highly reliable and cost-effective approach for SNP genotyping in large populations.

**Mutation analysis on Korean patients with tuberous sclerosis with the method of whole *TSC1* and *TSC2* cDNA sequencing.** *K.M. Hong*<sup>1</sup>, *Y.S. Kim*<sup>3</sup>, *Y.J. Woo*<sup>4</sup>, *W.Y. Jung*<sup>5</sup>, *C.H. Shin*<sup>2</sup>, *Y.B. Choi*<sup>2</sup>, *M.K. Paik*<sup>2</sup>. 1) Genetics Laboratory, Oregon Health Sciences University, Portland, OR 97201-3098; 2) Department of Biochemistry, Wonkwang University College of Medicine, Iksan, 570-749, Korea; 3) Department of Neurology, Wonkwang University College of Medicine, Iksan, 570-749, Korea; 4) Department of Pediatrics, Chonnam University College of Medicine, Kwang-Ju, 510-190, Korea; 5) Department of Neurology, Chosun University College of Medicine, Kwang-Ju, 501-759, Korea.

Tuberous sclerosis is an autosomal dominant neurocutaneous disorder with the prevalence of about 1:6,000. It is characterized by hamartomas in the skin, central nervous system, and the patients have epilepsy, mental retardation, facial angiofibromas, hypomelanotic patches and renal angiomyolipomas. It is a genetic disorder, but about 60% of the patients are sporadic form, apparently representing new mutations. This fact, along with the grave symptoms necessitates simpler screening methods. The disease is caused by the mutations in either of two large genes, *TSC1* and *TSC2*. These mutations are not clustered at some specific sites but are scattered over the entire gene. In this study, we have made a molecular diagnosis of tuberous sclerosis in five Korean patients by whole *TSC1* and *TSC2* cDNA sequencing. In each three patients, we found previously described mutations (R245X in *TSC1*, R611W and an exon 26 deletion in *TSC2*). In each of the other two patients, we found a 3-bp deletion in *TSC2* that might be a mutation. Additional studies will be performed to confirm the significance of these initial findings.

**A DHPLC protocol for detecting RET proto-oncogene mutations in MEN2 patients and medullary thyroid carcinoma.**

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Germline activating mutations of the RET proto-oncogene cause three different dominantly inherited cancer syndromes, including multiple endocrine neoplasia type 2A (MEN 2A), type 2B (MEN 2B), and familial medullary thyroid carcinoma (FMTC). Mutations, involving the somatic cell lineage, are found in about 30% of sporadic medullary thyroid carcinomas (MTC). Early detection of mutations is mandatory for genetic counselling and risk assessment in family members allowing presymptomatic testing and improvement of the disease management. The majority of the activating RET mutations affect exons 10, 11, 13, 14, 15 and 16 and are currently detected by SSCP and restriction enzyme analysis. In order to improve sensitivity, time and cost of the RET mutation analysis, we have developed a Denaturing High Performance Chromatography (DHPLC)-based protocol. In this system mutations can be determined on the basis of the melting behaviour of heteroduplexes, which elute from the column by a combination of temperature and acetonitrile gradient. We performed DHPLC in 141 MTC patients with previously characterized mutations and 35 relatives. Heteroduplex peaks were detected for each mutation tested which produced a distinct and highly reproducible DHPLC elution profile. These results indicated that DHPLC methodology: a) displays a high level of sensitivity, approaching 100% for mutations in the RET proto-oncogene; b) is suitable for rapid genetic testing of members of the MEN2 affected families; c) provides a relatively simple and accurate screening technique by exhibiting advantages over conventional mutation methods, including semi-automated analysis of 96 PCR samples in less than 12 hours and low cost .

**Six novel point mutations in the Dystrophin gene identified in Brazilian Duchenne patients.** *D.N.F. Vagenas<sup>1,2</sup>, A. Cerqueira<sup>1</sup>, A. Torres<sup>1</sup>, R. Pavanello<sup>1</sup>, M.R. Passos-Bueno<sup>1</sup>, M. Zatz<sup>1</sup>.* 1) Centro de Estudo Genoma Humano, Universidade de So Paulo, So Paulo, S.P, Brazil; 2) Centro em Interunidades em Biotecnologia, Universidade de So Paulo, S.P. Brazil.

Duchenne (DMD) and Becker (BMD) type muscular dystrophies are allelic X-linked conditions caused by mutations in the gene encoding dystrophin. This gene contains 79 exons, which are distributed in approximately 2,400 Kb. The phenotype is caused in about 60% of the cases by deletions, 5 - 6% by duplications, while the remaining cases are due to point mutations or small deletions or rearrangements. We have ascertained so far 1010 families with DMD patients in our center. Among them 632 had deletions in the dystrophin gene. We are currently trying to identify the mutations in the non-deleted cases through SSCP, PTT (Protein Truncation Test) and sequencing of abnormal fragments. For PTT analysis, the dystrophin gene was divided in 10 fragments, each one with 8 exons, encompassing the 79 exons. In 99 patients, about 4 fragments were randomly analyzed leading to the identification of 10 mutations. Among these, six are novel mutations: one deletion of exon 38, three frameshifting involving the exons: 5 (494-497 delAGTA); 16 (2067 - 2068 del TG and 2080 - 2083 del ACAA); and two nonsense in the exons 17 (2345 C@T; Q 713 X) and 45 (6785 T@C; Q 2193 X). We are also assessing the intellectual capacity in our patients in order to verify what mutations/deletions are more commonly associated with mental impairment. CEPID-FAPESP, PRONEX, CNPq, IAEA.

**One step and reliable full HFE genotyping using DHPLC methodology.** *S. Pissard, L.T.A. Huynh, J. Martin, M. Goossens.* lab biochemistry and genetics, hop henri Mondor, Creteil, France.

In Caucasian populations, genetic hemochromatosis is a frequent autosomal recessive disorder which results in an increased absorption of dietary iron. Iron over storage leads to severe disorders which may result in serious complications and life expectancy shortening. The product of the HFE gene (chromosome 6) is one regulator of iron uptake through interaction with the transferrin receptor. Two mutations, C282Y (exon 4) and H63D (exon2) are strongly associated with the disease. C282Y homozygous or C282Y / H63D compound heterozygous genotypes are now stated to have a diagnostic value in genetic hemochromatosis . Therefor, in addition to biochemical determination of serum iron, iron storage (ferritin) and transferrin saturation, HFE genotyping is one step of the hemochromatosis diagnostic. In most laboratories, genotyping is done by means of conventional PCR restriction assays which are very sensitive and specific but time consuming and finally, costly through consumption of reagents and working time. DHPLC is a new powerful and automated method which is now a widely used tool for SNP detection. However, a main limitation of this method is that in some instance, it would not discriminate between homozygous wild type and homozygous mutant DNA. This makes its use difficult in genotyping for recessive disease. To override this limitation, we have designed a simple multiplex ARMS PCR which allowed to detect simultaneously these two mutations. Crude, unlabelled PCR products are run in non denaturing conditions (45C) giving unambiguous chromatogram patterns for wild type, mutant and heterozygous alleles. Primers were designed to bracket regions in HFE exon2 and exons 4 where other rare HFE mutations linked to hemochromatosis have been described, as the S65C mutation in exon 2. Running the same PCR products in denaturing conditions (58C) allows the diagnosis of these rare mutations even if the chromatogram pattern becomes more complex. This strategy greatly improve the usefulness of DHPLC making its advantages (full automation, low cost unlabelled PCR, fast method) available for genotyping in genetic diagnostic laboratories.

**Resolution of equivocal molecular genetic test results in the diagnosis of Facioscapulohumeral Muscular Dystrophy (FSHD).** *W.K. Seltzer<sup>1</sup>, K. Felice<sup>2</sup>, J. Durocher<sup>1</sup>, A.R. Yesley<sup>1</sup>, M.A. Boss<sup>1</sup>, S.E. Hallam<sup>1</sup>.* 1) Athena Diagnostics, Inc, Worcester, MA; 2) University of Connecticut Health Center, Farmington, CT.

Ninety to 95% of Facioscapulohumeral muscular dystrophy (FSHD) cases are associated with a deletion within a repeat array. Athena Diagnostics, Inc. performs FSHD analysis by EcoRI and EcoRI/BlnI restriction endonuclease digestions followed by Pulsed-Field Gel Electrophoresis to separate and resolve the 8 to > 48 kb sized DNA fragments. Southern analysis is then performed using probe, p13E-11, which hybridizes to repeat arrays at both 4q35 and 10q26. BlnI specifically digests the chromosome 10 repeat array to small fragments allowing discrimination between chromosome 10 and chromosome 4 arrays. Twenty percent of the general population carry a benign translocation resulting in either three chromosome 4q35 repeat arrays and a single 10q26 array, or alternatively, three 10q26 arrays and a single 4q35 array. This complicates result interpretation since it is the location of the repeat array deletion, and not its chromosomal origin, which determines the pathogenicity of the deletion. The presence of these translocations can potentially lead to false positive and false negative test results. We reviewed 275 cases of which 49 (18%) yielded findings that could not be unequivocally interpreted. In order to achieve a better understanding of the remaining equivocal results, we carried out XapI analyses which specifically digest the chromosome 4 repeat array. This provided significant utility in interpretation of these previously equivocal results.

**Frequency of Y-chromosome microdeletion in a non-selected population.** *G. Massazza, M. Poma, M. Iascone, U. Giussani, D. Chinaglia, A. Parma.* Clinical Pathology, Ospedali Riuniti di Bergamo, Bergamo, Italy.

Recent studies have indicated that microdeletions of AZF region in Yq could affect the normal expression of spermatogenesis factors. Every year we perform 1750 semen analysis most of which concerning men with fertility impairment. We decided to combine standard morphological analysis with PCR screening of microdeletions of the Y to assess if a sperm count  $< 5 \times 10^6$  spermatozoa /ml could be a parameter sufficient to justify the molecular analysis. We also wanted to correlate the clinical phenotype of infertile men with specific Yq-microdeletions. 2537 semen were analysed according to WHO guidelines (1999) indicating 90 azoospermic, 425 oligozoospermic and 2022 normozoospermic subjects. The patients mean age was 33 years (range 15-62). Genomic DNA was extracted from 193 azo-oligozoospermic and 15 normozoospermic semen samples and from peripheral blood lymphocytes (PBL) of 36 men enrolled in the protocol for assisted reproduction (mean age: 33yr range 25-38). Performing 2 multiplex-PCR in semen specimens we found microdeletions in 10,6% of azoospermic subjects and in 3,4% oligozoospermic men. An azoospermic patient showed deletion of AZFc region. Histological examination on testicular biopsy diagnosed a Dysgenetic Sertoli-cell only syndrome (SCOS). Another azoospermic patients had all three AZF loci missing. The following cytogenetic analysis revealed the presence of an isodicentric Y chromosome and the karyotype resulted 46,X, ish idic (y)(q11)(DYZ3++,DYZ1-). 34/36 azo-oligozoospermic men enrolled to assisted reproduction protocol had 46,XY karyotype, one was 46,XX male and one had a 46, XY (t13:14). Molecular analysis of specific Yq-STS on PBL revealed 4/5 oligozoospermic men deleted in AZFb regions. Our findings indicate that cytogenetic analysis and molecular screening of Y-chromosome microdeletions are necessary tools helping the diagnostic process in male infertility and they should be advise especially in those patients with sperm concentration  $< 5 \times 10^6$ /ml.



**Cytochrome P450 2D6 isoenzyme genotyping using derivative melting curve analysis on the LightCycler. A.**

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Genetic polymorphism of the cytochrome P450 superfamily results in phenotypic variation in drug metabolism that can affect therapeutic responses. CYP2D6 is an isoenzyme in this family that catalyzes the oxidative biotransformation of many commonly prescribed drugs including selective serotonin reuptake inhibitors, tricyclic antidepressants, antiarrhythmics,  $\beta$ -receptor blockers and neuroleptics. CYP2D6 polymorphism results in several phenotypes, including poor (PM), extensive (EM or wild type) and ultra (UM) metabolizers. Two variant alleles of the CYP2D6 gene, CYP2D6\*3 (A2637 deletion) and CYP2D6\*4 (splice site mutation G1934A) account for 70% of poor drug metabolizers. A deletion of the gene, CYP2D6\*5(del), also results in a poor metabolizer phenotype and occurs in approximately 2% of the Caucasian population. These polymorphisms are considered autosomal recessive inheritance.

Detection of the \*3, \*4, \*5 alleles has been developed using rapid PCR and melting curve analysis on the LightCycler®. Primer sets were designed to amplify two specific regions of the gene. The temperature at which hybridization probes dissociate from the template distinguish the wild type and polymorphic alleles. Comparison of derivative melting curve areas of CYP2D6 with the area of an internally amplified reference gene,  $\beta$ -globin, characterizes the gene. The normalized area ratio of  $\beta$ -globin to CYP2D6 for a normal gene dose ranges from 0.76-1.24 with a 99% confidence interval. Gene deletions have ratios >1.5. Allele frequencies determined using this method are 2.6% for CYP2D6\*3 and 22.8% for CYP2D6\*4 (n=114). Allele frequencies for CYP2D6\*5(del) are 0.9%. Gene dose analysis by derivative melting curves, represented by the CYP2D6\*5 system, is a methodology that can be widely applied to detect chromosomal duplications and deletions.

**Prenatally diagnosed neural tube defect rates before and after food fortification in California.** *L. Feuchtbaum, R. Currier, M. Kharrazi, F. Lorey, G. Cunningham.* Genetic Disease Branch, California Dept Health Services, Berkeley, CA.

Since 1992 when the Public Health Service announced that neural tube defects (NTDs) could be reduced by 50% if all women of reproductive age consume 400 micrograms of folic acid daily, there has been a public policy debate about how to achieve this goal. In 1996, the FDA mandated that by January 1, 1998 all cereals, breads, and other foods labeled as enriched be fortified with 140 micrograms of folic acid per 100 grams of cereal. In California, the impact of food fortification on NTD prevalence has yet to be examined. This study presents changes in the prevalence of newly diagnosed, prenatally detected NTDs, for the three-year period before and after the food fortification deadline.

The Genetic Disease Branch runs the California Expanded AFP Screening program to screen for NTDs and selected chromosome abnormalities in the 2nd trimester of pregnancy. The program diagnoses about 80% of all NTDs statewide. Women with screen-positive test results are referred for follow-up at a state-approved Prenatal Diagnosis Center that reports counts of diagnosed cases quarterly. This study presents quarterly NTD diagnosis rates (per 1000 referred women) from 1995 through 2001.

To date, 1,316 NTDs were diagnosed among 120,420 referred women including 680 anencephaly, 452 spina bifida, and 99 encephalocele cases. Overall, Hispanics had the highest anencephaly rate and Whites had the highest spina bifida rate. The overall NTD rate for the period after the fortification deadline declined by 24% ( $p < 0.01$ ). However, statistically significant declines were only observed among Whites. The anencephaly rate declined about 28% ( $p < 0.01$ ) and statistically significant declines were observed among Whites and Hispanics. The spina bifida rate declined about 12% ( $p = 0.17$ ) and the encephalocele rate declined about 11% ( $p = 0.55$ ). The regression line for NTD rates before the fortification deadline was much steeper than the line after the deadline indicating that rates appear to have leveled off in the past three years. The implications of these and other findings are discussed.

**Cost Effectiveness of tandem mass spectrometry (MS/MS) for neonatal screening: Medium chain acyl-CoA dehydrogenase (MCAD) as a model.** *D.N. Finegold<sup>1</sup>, E.W. Naylor<sup>2</sup>, D.H. Chace<sup>2</sup>, M. Kamlet<sup>3</sup>*. 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Neo Gen Screening, Inc, Pittsburgh, PA; 3) H. John Heinz III School of Public Policy and Management, Carnegie-Mellon University, Pittsburgh, PA.

Newborn screening is an accepted component of preventative public health measures. The acceptance of a test for newborn screening is dependent on incidence of disease, simplicity of testing, effective treatment for the disease to be tested, and considerations of economic costs. Recent technological advances such as MS/MS have provided alternative methods besides bacterial inhibition assays (BIA) to screen newborns for PKU and other metabolic diseases. MS/MS is able to rapidly detect over twenty inborn errors of metabolism in addition to PKU with little incremental cost. We performed a cost effectiveness analysis (CEA) of newborn screening assuming MS/MS as a screen for both PKU and MCAD, compared to using BIA to screen for PKU. Initial studies showed BIA for PKU alone, excluding the costs of follow-up for false positive tests, to be more cost effective than MS/MS for PKU. We then modeled MS/MS screening for MCAD since BIA testing will not detect MCAD. We included estimates of deaths caused by MCAD, children damaged by metabolic episodes caused by MCAD, children surviving to adulthood without an episode caused by MCAD, the estimated incidence of MCAD, costs of screening, as well as other factors. Based on dollars-per-quality-adjusted-life-years (\$/QALY), MS/MS is a cost effective screening tool to detect newborns at risk with MCAD. Since the added costs of screening for both MCAD and PKU are negligible, we concluded that MS/MS is a cost effective screening tool compared to BIA for PKU, and not screening for MCAD. MS/MS screening is able to detect many more disorders than MCAD and PKU. Further studies are ongoing to determine the overall cost effectiveness by \$/QUALY of MS/MS as a tool in newborn screening including all diseases detectable by MS/MS as well as cost of follow-up.

**Consequences for health care facilities if guidelines for healthy women with breast cancer in family history are applied.** *C.J. Asperen van*<sup>1</sup>, *R.A.E.M. Tollenaar*<sup>2</sup>, *E.M.M. Krol-Warmerdam*<sup>1</sup>, *F. Erp van*<sup>5</sup>, *C. Seynaeve*<sup>5</sup>, *P. Devilee*<sup>1,3</sup>, *C.J. Cornelisse*<sup>3</sup>, *C.T.M. Brekelmans*<sup>5</sup>, *J.G.M. Klijn*<sup>5</sup>, *G.H. Bock de*<sup>4</sup>. 1) Dept. Human and Clinical Genetics, Leiden University Medical Center Leiden, The Netherlands; 2) Dept. Surgery,; 3) Dept. Pathology,; 4) Dept. Medical Decision Making,; 5) Dept. Medical Oncology, Daniel den Hoed Cancer Center, University Hospital Rotterdam.

**Background** Many studies found an increased risk to develop breast cancer for first and second-degree relatives of breast cancer patients. This study analyzed the consequences for health care facilities if guidelines for advising healthy women with breast cancer in family history are applied.

**Methods** In a hospital based cohort of recently diagnosed breast cancer patients, 891 family histories were analyzed. These were obtained by interview and questionnaires and contained information about family size, first and second-degree relatives, age and cancer diagnosis. The schemes of the Dutch and British guidelines were applied on the family histories, by using SPSS 10.

**Results** In 891 families 3303 female first-degree relatives were reported 2021 (62%) were alive, above the age of 24, and unknown in a genetic department. Of this last group 672 (33%) women were between 35 and 50 years. When applying the guidelines for referrals, it was calculated that for each case of breast cancer 0.3 first degree female relative would be offered a consultation or a referral to a clinical genetic service. Likewise, per breast cancer case, 0.3 first-degree female relative will be offered a current advice for breast surveillance according to the Dutch guideline and 0.1 according to the British guideline.

**Conclusion** If available guidelines for healthy relatives of breast cancer patients are fully applied, this will lead to a sharp increase in the use of health care facilities. Scientific advances in breast cancer genetics combined with the growing public interest in breast cancer prevention could meet economic limitations. Further research is needed to focus on the cost-effectiveness of these guidelines.

**An individual and population-based framework for improving the public's health and wellbeing--with special emphasis on genetics and genetics-related issues.** *R.M. Fineman<sup>1</sup>, M. Puryear<sup>2</sup>, C. Crain<sup>3</sup>, P. Kyler<sup>2</sup>, J. Massad<sup>3</sup>, B. Oles<sup>3</sup>, C. Roeber<sup>3</sup>, N. Tashima<sup>3</sup>.* 1) Department of Health Services, UW School of Public Health, Seattle, WA; 2) GSB,MCHB,HRSA,DHHS,Rockville, MD; 3) LTG Associates,Takoma Park, MD.

Using a medical model in 1994, Evans and Stoddart proposed a relational framework to describe "the diverse determinants of health, as well as to permit a definition of health broad enough to encompass the dimensions that people--providers of care, policymakers, and particularly ordinary individuals--feel to be important." We have built upon this framework to create an updated version of this model that better defines and describes, from a public health (PH) genetics perspective, why some people enjoy good health and wellbeing, while others do not. Our model includes multiple health and health care determinants (e.g., the interaction of genetic, environmental and social risk and protective factors, access to care, etc.); the MCHB/HRSA/DHHS "pyramid" that includes direct patient services, enabling services, population-based services, and infrastructure/capacity-building services; governmental investment opportunities that influence health, health care, and wellbeing; and informed decision-making by individuals, families, and communities. Our model also connects PH genetics theory to practice, and it is analytical enough (e.g., goals, objectives, performance measures, guidelines, etc.) to measure health, wellbeing, and health promotion and disease prevention activities well into the 21st century--especially as they pertain to genetics and genetics-related issues.

**Results of a national survey re: retention and maintenance of clinical genetics records.** *W.S. Meschino.* Dept Genetics, North York General Hosp, Toronto, ON., Canada.

Issues related to the retention and maintenance of clinical genetics records may differ from those of the general health record because of the very nature of genetic information. Results of genetics consultations and tests relate not just to individuals, but to families, and have potential significance for future generations. Most genetics departments across Canada have been in existence less than 30 years. Over time, constraints of space and resources to maintain records may increasingly influence genetics departments to consider whether records should be destroyed as per provincial regulations for hospital/medical records, stored elsewhere or integrated into the main health record of the institution. A review of the relevant medical literature and provincial regulations in several provinces was conducted, and 21 genetic centres across Canada were surveyed. 18/21 centres returned completed questionnaires. Almost all centres (17/18) routinely maintain clinical genetics records indefinitely and believe that this practice should continue, assuming adequate space and resources. Some centres (4/18) destroy a portion of the record after the regulation time period (usually routine prenatal cases) or maintain only part of the chart (3/17) for longer than required. In most centres, clinical genetics records are maintained separately from the general health record, but in 2/17 centres, at least a portion of the genetics records (e.g. consultation) is copied to the general health record of the institution. The vast majority (17/18) believe that clinical genetics records should be maintained separately from the general health record for the following reasons: 1. To maintain confidentiality especially considering the sensitive nature of the information. 2. Most genetics records represent families, not just individuals, and detailed family information/records are not appropriate as part of an individual's health record. However, genetics units should be responsible for informing their institution's health records department of the existence of a patient record in genetics. Electronic data storage, microfilm etc. may be required to address ongoing space needs.

**Consumer interest in genetic testing for breast cancer susceptibility: Personal decision-making model. E.L.**

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Genetic testing for breast cancer risk will soon extend beyond rare, high-risk genes, such as deleterious mutations in BRCA1/BRCA2. Also, there is evidence that risks associated with such mutations may be lower than estimated from high-risk families. Anticipating new knowledge about genetic susceptibility to breast cancer, we assessed consumer opinions about genetic testing for breast cancer risk from a broad perspective (rare, high-risk genes; more common, moderate-risk genes). A survey (Genetic Testing and Breast Cancer: Your Opinions) was completed by 303 adult female KP Northwest health plan members. Survey content was based on a personal decision-making model of genetic testing. Respondents were predominantly white non-Hispanic (77%); median age was 59 years. Factor analyses showed several factors for personal impact of testing, several related to breast cancer screening, and self risk perception. Univariate analyses of factor scores and interest in genetic testing suggest that interest is highly related to intention to change screening and prevention behaviors, greater personal control with knowledge of test results, importance of breast cancer screening, and open access to testing. Interest was not related to self risk perception, intended family disclosure or concerns, worry about test results, current breast cancer screening, or general mental health. Previously, we reported that gene and test characteristics were related to interest. Results from multivariate analyses, integrating information from all aspects of the personal model, will be available for presentation. Consumer preferences should be considered by policymakers when recommending strategies for genetic testing.

Supported by NCI P01 CA72085 and PHS T32 HS00069.

**Newborn Screening by Tandem Mass Spectrometry for Medium Chain Acyl-CoA Dehydrogenase Deficiency: Is**

**it Cost Effective?** *C.P. Venditti<sup>1</sup>, L.N. Venditti<sup>3</sup>, C.A. Stanley<sup>2</sup>, G.T. Berry<sup>1,2</sup>, P. Kaplan<sup>1</sup>, E.M. Kaye<sup>1</sup>, H. Glick<sup>3</sup>.* 1)

Division of Human Genetics and Molecular Biology and; 2) Department of Endocrinology, Childrens Hospital of Philadelphia,; 3) Department of General Internal Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA.

Tandem mass spectrometry (MS/MS) has been advanced as a new technology that can be used to effectively screen for several classes of inborn errors of metabolism that are not detected by conventional newborn screening modalities. The cost-effectiveness of using this method has not been fully explored. To begin to address this problem, we have employed decision-analytic modeling to examine whether newborn screening by MS/MS for a relatively common and treatable fatty acid oxidation disorder, medium chain acyl-CoA dehydrogenase deficiency (MCAD), is cost-effective. We developed a computer model to evaluate the incremental cost per quality-adjusted life year (QALY) saved of newborn screening for MCAD compared to not screening. A hypothetical cohort of neonates was followed from birth using a Markov simulation model. In this model, subjects made transitions among a set of health states that reflected clinical status, morbidity, and cost. Outcomes were estimated for 3 time horizons, 10, 20 and 70 years. Transition probabilities were derived from empiric clinical data. Probabilities of clinical events and average Markov state costs were derived from chart reviews of a thirty-two patient cohort treated over the past thirty years, patient-family interviews, national cost surveys, and clinical experience with MCAD patient management. Estimates of the expected net changes in costs and life expectancy for MCAD screening were used to compute the incremental cost-effectiveness ratio (ICER) as compared to not screening. The 20-year incremental cost-effectiveness ratio (ICER) of newborn screening for MCAD is within the acceptable range of <\$50,000 US dollars per quality-adjusted life year gained; even with an overestimated screening cost and simulating only 10 years of life, the ICER is in the \$50,000/QALY zone. These results argue for the widespread implementation of newborn screening for MCAD.



**Detection of Rare FGFR3 alleles in sperm samples using the Invader Operating System.** ©. *M.W. Baker<sup>1</sup>, D. Lind<sup>2</sup>, D. Cox<sup>2</sup>, L. Wang<sup>1</sup>, B.P. Neri<sup>1</sup>, M. de Arruda<sup>1</sup>*. 1) Third Wave Technologies, Madison, WI; 2) Dept. of Genetics, Stanford University School of Medicine, Stanford, CA.

Mutations in the Fibroblast Growth Factor Receptor 3 (FGFR3) gene can cause Achondroplasia, the most frequent form of short-limb dwarfism. Achondroplasia is an autosomal dominant disorder; a majority of cases are sporadic, the result of a de novo mutation. It is estimated that more than 95% of the cases of Achondroplasia are associated with a G-to-A transition at nucleotide 1138 of the FGFR3 gene, while the second most common mutation is a G-to-C transversion at the same position. Based on previously published studies detailing prevalence (birth rate), it is predicted that the FGFR3 mutations occur at an estimated rate between 1.72 and  $5.57 \times 10^{-5}$  per gamete per generation.

Here we report the use of the Invader Assay to detect the presence of mutant G1138A and G1138C FGFR3 alleles in human sperm samples. The Invader Operation System is an isothermal, homogenous genotyping assay that uses fluorescence energy transfer (FRET) as a detection method. The unique specificity of the Invader Assay permits discrimination of mutant and wild type alleles at a resolution of at least 1:1,000.

We have used pools of about 1,000 sperm to amplify a region of 0.4 kb of the FGFR3 gene, which contained the 1138 locus. The amplification product was then used in the Invader Assay. Preliminary results from approximately 120 sperm pools, have detected the presence of mutant G/A allele in 5 pools and the presence of the G/C mutant allele in only of them. Combined together, the occurring frequency of the two mutants is very close to the birth rate of Achondroplasia in the general population. These results indicate that the Invader assay offers a simple and highly efficient method for screening sporadic mutations in a background of wild type sequence.

**Evaluation of the probe specificity used in INNO-LiPA CFTR12: No false positives with polymorphisms I506V, I507V, F508C, and I506M.** *P. Hendrix, C. Van Loon, I. De Canck.* Innogenetics, Gent, Belgium.

The INNO-LiPA CFTR12 and INNO-LiPA CFTR17 + Tn respectively identify 12 and 17 specific CF-related mutations and their wild-type sequences, as well as CBAVD-related polymorphism Tn in intron 8. The technology used is a simple reverse hybridization of an amplified product on a nitrocellulose strip carrying specific oligonucleotide probes as parallel lines. The amplified product is the result of an optimized multiplex amplification. Hybridization and stringent wash occur at the same temperature and can be performed either manually in a water bath or using the Auto-LiPA. Both assays were successfully validated in a European multicenter study and are suited for DNA extracted from whole blood and buccal brushes. Also, DNA extracted from amniotic fluid, chorionic villi, and dried blood spots has been shown to be compatible with the assays in exploratory evaluations. Individuals, homozygous for a mutant or a wild-type allele will only hybridize with the corresponding probe, whereas individuals heterozygous for a particular mutation will hybridize with both the mutant and wild-type probe for this mutation. The ACMG recommendations for General Population Carrier screening for CF emphasize the risk of unexpected homozygosity for DF508 and/or DI507, due to benign variants at codons 506, 507, and 508 in the CFTR gene. Therefore, we undertook to test the INNO-LiPA CFTR assay specificity for samples containing either the I506V, I507V, F508C, and even the I506M polymorphism. These sequence variants were introduced by mutagenesis and controlled by sequence analysis. In addition, three clinical samples with proven presence of F508C and linked with the S1251N mutation were included. None of the samples tested showed any false positive reactions with the mutant probe for DF508 and/or for DI507; in all cases, the wild-type probe for DF508/DI507 remained positive, allowing a correct clinical interpretation.

**Evaluation of a supratypic HLA-genetic marker for susceptibility to chronic beryllium disease: racial variation has no significant impact for pre-employment screening.** A. Weston, J. Ensey, K. Kreiss, C. Keshava, E. McCanlies. National Institute for Occupational Safety and Health, CDC, Morgantown, WV.

Codon 69 of *HLA-DPBI* is tri-allelic (AAG - lysine [K], GAG - glutamic acid [E] and AGG - arginine [R]), where the relative frequencies are K>E>R. The lysine allele is found in 54 of 88 known variants of this gene, glutamic acid is found in 29 and arginine in 5. Three published studies report an association of the supratypic marker *HLA-DPB1E69* with susceptibility to disease following occupational exposure to beryllium, where odds ratios range between 12 and 76 (95% CIs range = 2-322). Consequently, testing for *HLA-DPB1E69* is currently being considered as a pre-employment screening tool. This study investigates the prudence of this course of action in light of calculations of the positive predictive value, sensitivity and specificity of this supratypic marker in four racial populations. Polymerase chain reaction and restriction analyses were used to determine *HLA-DPB1E69* population frequencies. Calculation of positive predictive values assumed a disease frequency among beryllium workers of 5% for the workforce in general. Selection of an odds ratio for disease risk of 35 for the HLA marker was based on analysis of data previously reported in the literature by others. Allelic/carrier frequencies were found to be 0.21/0.33, 0.24/0.40, 0.27/0.47 and 0.38/0.59 for Caucasians, African-Americans, Latinas and Chinese respectively. Ranges of positive predictive values for a genetic test based on *HLA-DPB1E69* in these populations were calculated to be 8.3 - 14.3% for carriers with an assumed disease prevalence of 5%. These estimates suggest that using *HLA-DPB1E69* genotyping for general pre-employment screening in the beryllium industry has a low positive predictive value, regardless of the fact that there are significant differences in carrier frequencies among racial groups.

**Screening for the alpha 1-antitrypsin deficiency (a1ATD) mutation PiZ in Israeli Jewish populations. An apparent another frequent Ashkenazi-Jewish disorder.** . V. Adir, E. Golinker, E. Sachak, T. Falik-Zaccari, Z. Borochowitz. The Simon Winter Institute for Human Genetics, Bnai-Zion Medical Center, Technion-Rappaport Faculty of Medicine, Haifa, Israel.

a1ATD is a severe incurable hereditary autosomal codominant disorder. The homozygous PiZZ genotype accounts for all a1ATD childhood cases of liver disease and early onset of pulmonary emphysema. Being a hereditary fatal disorder with a known, simple to identify molecular genotype which accounts for all affected individuals, it thus fulfills the WHO criteria for population genetics screening tests. In order to justify a1ATD as a suitable disorder to be screened in our population, we needed to determine the frequency of the disease alleles responsible for all severe cases of a1ATD among the various ethnic groups in our targeted population. A total of 4226 Israeli Jews were divided into 3 ethnic groups: Ashkenazi (both parents are Ashkenazi n=2683), non-Ashkenazi (n=983) and 'mixed' origin (Ashkenazi with non-Ashkenazi, n=560), and were tested for the presence of the PiZ allele, using DNA-based analysis. Of 33 carrier individuals of the PiZ allele, 29 were Ashkenazi, and 4 were from the 'mixed' group, giving rise to a carrier frequency of 1:92. The frequency of the PiZ allele among the 'mixed' group was 1:140 which was higher than expected, however may be accounted to the small sample size. These results resemble the frequency of carriers found in European populations, however they are 6 fold higher than that shown by Nevo and Cleve (1). In their biochemical analysis of 550 Ashkenazi-Jews, only a single carrier was found. The discrepancy between our results and those determined by Nevo and Cleve can be explained by our larger sample size and the greater accuracy of DNA based techniques. Due to the higher prevalence of the PiZ allele found among the Ashkenazi Jewish population, along with the carrier frequency of 1 in 92 in that group, we strongly recommend screening the Ashkenazi Jewish population also for the PiZ allele. 1)Nevo S. and Cleve H: *Am. J. Med Genet* 1991; 15;39(4):399-403.

**A highly sensitive fluorescence-based maternal cell contamination test.** *W.C. Spence<sup>1</sup>, C.M. Kelly<sup>1</sup>, A. Maddalena<sup>1,2</sup>.* 1) Genetics & IVF Institute, Fairfax, VA; 2) Medical College of Virginia, Richmond.

The misinterpretation of a fetal molecular result can occur in pregnancies at high risk for a specific genetic disease if a significant percentage of maternal cells are present in the fetal specimen. Maternal cells in products of conception (POC) or other fetal cultures also have the potential to lead to an erroneous fetal interpretation when doing cytogenetic analysis. To aid in the interpretation of these molecular and cytogenetic cases, the GIVF molecular laboratory has modified a test that is being widely used in the paternity and forensics community for identity testing. The AmpFeSTR®Profiler Plus™ kit examines nine independent highly polymorphic loci. The primers are fluorescently labeled and the amplified product is analyzed by capillary electrophoresis on an ABI Prism® 310. Because of the polymorphic nature of the tested loci, the mother generally has two different alleles at many of the nine loci. One of these alleles is inherited by the fetus. If the second maternal allele is not shared by the fetus, this locus is considered to be informative. There is evidence of maternal cell contamination (MCC) if any amount of a second maternal allele in an informative system is detected in the fetal sample. Mixing experiments have been done to determine the sensitivity of this MCC fluorescent test. Contaminating DNA can begin to be detected at a level of approximately 1%. Conservatively, this fluorescent test can always detect contaminating DNA when it is present at a level of 10% or more. We have run mixing experiments on several of our molecular genetic tests and verified that 10% DNA contamination does not lead to a misinterpretation of the results. Therefore, this MCC test has the sensitivity needed to verify that a molecular test result is reliable in a fetal sample. It will also be useful in detecting MCC in fetal cytogenetic cases and may be particularly valuable when karyotyping POC.

**Minimization of the Time and Cost to Identify Unknown RB1 Mutations.** *K.E. Vandezande<sup>1</sup>, S. Richter<sup>1</sup>, N. Chen<sup>1,4</sup>, K. Zhang<sup>1</sup>, X.C. Tong<sup>1,3,4</sup>, B.L. Gallie<sup>1,2,3,4</sup>.* 1) Solutions by Sequence Inc, Toronto, ON; 2) Princess Margaret Hospital, Toronto, ON; 3) Ontario Cancer Institute, Toronto, ON; 4) Canadian Genetic Diseases Network.

Despite clear economic and clinical benefits, RB1 mutation detection is not widely implemented in care for retinoblastoma families. Testing for RB1 mutations is difficult and relatively expensive. The RB1 gene is large (180kb); as with other tumor suppressor genes (BRCA1, eg), a variety of mutations can disrupt function, including whole gene deletions, partial deletions and point mutations. There are no common founder mutations and no particular hotspots for mutations. Thus, a series of tests is required to attain high sensitivity in clinical mutation identification. We use 6 quantitative PCR multiplexes to detect deletions and insertions and 14 automated sequence runs (2 exons each) to detect substitutions. We developed general rules to find the most cost-effective order for molecular tests and applied them to the search for RB1 mutations. Based on mutations found for 302 of 349 retinoblastoma probands (86% sensitivity), we found the optimal order for finding RB1 mutations. Mutation discovery rates in sequencing exons are independent of test order, so sequencing tests rank optimally on a simple bang-for-buck measure. Mutation discovery rates from quantitative multiplex PCR depend on the order in which multiplexes are performed, so we examined all permutations of the 6 multiplexes to find the most effective. Last, all 20 tests were ordered by decreasing cost effectiveness to achieve an optimal test order. To find an unknown RB1 germline mutation, the naive strategy (all quantitative analyses before sequencing 14 duplexes) takes 125 hours and costs \$3,436(CAD). After optimization, the search is predicted to take an average of 117 hours and to cost \$2,951. Optimizing RB1 mutation detection decreases costs and turnaround time and increases the likelihood of clinical implementation. Our search algorithm is applicable to other genetic diseases and to general cost impact assessment of new techniques before implementation.

**Rapid and specific detection of b-thalassemia mutations in Malaysia using Combine-ARMS.** *J.A. Tan, K.L. Tan, Y.C. Wong, Y.C. Wee, S.F. Yap.* Allied Health Sci, Fac Med, University of Malaya Medical Centre, Kuala Lumpur, Malaysia.

The percentage of compound heterozygosity found in b-thalassemia major patients in the University of Malaya Medical Centre (UMMC) is about 47.62%. Thus, much time and effort is spent in the detection of two b-mutations per family in the event of a prenatal diagnosis. A Combined-ARMS (C-ARMS) technique was established for the detection of two to three b-mutations in a single PCR reaction. C-ARMS protocols for cd 41-42/IVSII #654 and -29/cd 71-72 detected b-mutations in 74.98% of the Chinese b-thalassemia patients in UMMC. C-ARMS for cd 41-42/IVSI #5/cd 17 detected b-mutations in 36.53% of b-thalassemia in the Malay patients. We conclude that Combine-ARMS, with the ability to detect two to three specific b-mutations in a single PCR reaction, provides a more rapid and cost-effective technique for prenatal diagnosis and molecular characterization of b-thalassemia in Malaysia.

**The experiences of a predictive testing programme for cancer genetics in South Africa.** *U.F. Algar<sup>1</sup>, P.A. Goldberg<sup>1</sup>, G.E. Pietersen<sup>2</sup>, J.A. Gilfillan<sup>2</sup>, R.S. Ramesar<sup>2</sup>.* 1) Colorectal Surgery, Groote Schuur Hospital, Cape Town, Western Province, South Africa; 2) Human Genetics, University of Cape Town, Cape Town, South Africa.

Research geneticists in South Africa have identified several novel mutations in the DNA mismatch repair genes underlying hereditary non-polytopic colon cancer (HNPCC). This has given clinical medicine an additional tool, namely predictive genetic testing, which can identify apparently healthy persons at high risk for developing predominantly colon cancer, later in life. Since 1990 genetic research bloods have been taken from 209 individuals of one HNPCC family (529 individuals on the pedigree). The majority of these family members live in a remote area in South Africa. As a result of a protocol of recruiting all individuals who are diagnosed with colon cancer under the age of 45 to an ongoing research programme, 10 families have been identified who have the identical genetic mutation. To date 440 predictive test results have been made known to these individuals, and of these, 21.36% are positive. Mutations detection in all these families has an impact on the psychosocial health of every individual involved, but it has also markedly reduced the burden of disease on the different health services (rural as well as urban, private and state) that are involved in delivering a service to these communities. Predictive testing in cancer genetics raises a number of questions for the spectrum of stakeholders associated with predictive testing: Are apparently healthy persons interested in knowing that they are at an increased risk of a particular cancer? How does this knowledge affect their wellbeing? Does knowledge of definitive risk status have an impact on society? What is the effectiveness of this "preventative" care? Does a tertiary health service have a duty to deliver health care? We will focus on these issues that have arisen in our service to HNPCC families in South Africa.



**What genetic issues do family physicians encounter?** *L.S. Acheson, S.J. Zyzanski, K.C. Stange.* Department of Family Medicine, Case Western Reserve Univ, University Hospitals of Cleveland, Cleveland, OH.

**Objective:** To describe the genetics-related clinical issues encountered by practicing family physicians (FPs), and which medical problems they are likely to refer to genetic consultants. **Methods:** Mailed questionnaire to a national, random sample of practicing FPs. FPs reviewed 18 categories of medical conditions and indicated how many times in the past year they had discussed genetic information about each of these conditions with patients, and what proportion of the families with each condition were referred for genetic consultation. **Results:** The response rate was 38%. Respondents were similar to nonrespondents except that more were women. 41% of the 190 physicians graduated from medical school before 1980. 47% practice in rural communities or small towns. 37% provide prenatal care. The majority of FPs had discussed the genetics of common cancers, cardiovascular disease (CVD), and Alzheimer's disease with 2 or more patients in the past year. 13% had referred patients for genetic counseling regarding breast-ovarian cancer but only 1 had referred a family because of CVD and 1 for Alzheimer's Disease. 25% to 50% of FPs had addressed genetic issues in at least one family with hemoglobinopathy, a blood clotting disorder, hemochromatosis, mental illness, vision loss or deafness, chromosome abnormality, infertility or pregnancy loss, congenital anomalies, mental retardation, and neurofibromatosis. Most cases where genetic issues were addressed were not referred for genetic consultation at that time. 23% of respondents said that genetic consultation is very difficult to obtain or unavailable. 17% listed ethical and social dilemmas that arose when considering whether to pursue genetic diagnosis. Lack of locally available consultants, worry about harms of testing, cost, and patients' opposition to abortion were the most frequently mentioned barriers to referral. **Conclusions:** Nationwide, family physicians are addressing a variety of genetic issues with patients. Genetic consultation is not currently used for some common adult disorders; it is more common for familial cancers and perinatal conditions. These data may be used in planning education and outreach to FPs.

**External Quality Assessment: A model scheme for Friedreich ataxia testing.** *D.E. Barton*<sup>1</sup>, *F. Ryan*<sup>2</sup>, *E. Sistermans*<sup>3</sup>, *M. Claustres*<sup>4</sup>, *L. Florentin*<sup>5</sup>, *S. Cocozza*<sup>6</sup>, *S. Patton*<sup>7</sup>, *R. Elles*<sup>7</sup>. 1) National Centre for Medical Genetics and University College Dublin, Ireland; 2) Dublin Institute of Technology, Dublin, Ireland; 3) University Hospital Nijmegen, Nijmegen, The Netherlands; 4) Institut de Biologie, Montpellier, France; 5) Lito Maternity Hospital, Athens, Greece; 6) University Federico II, Naples, Italy; 7) European Molecular Genetics Quality Network, Regional Molecular Genetics Laboratory, Manchester, UK.

Studies of the reliability of molecular genetic testing have indicated a significant level of inaccuracy, arising from errors in sample identification, genotyping or interpretation. The European Molecular Genetics Quality Network (EMQN) aims to raise and maintain the quality of molecular genetic testing by providing external quality assessment (EQA) schemes to diagnostic laboratories. EQA schemes are designed to test participants' ability in both genotyping and interpretation. EMQN has provided 15 EQA schemes in 6 disease-specific areas since 1997. We describe the results of an EQA scheme for Friedreich ataxia testing, and the development of best practice guidelines using the experience gained. Friedreich ataxia (FRDA) is the most common hereditary ataxia with an estimated prevalence of 1 in 50,000. The most frequent genetic abnormality seen in FRDA is the homozygous expansion of a GAA repeat in the FRDA gene. In 1998, EMQN organised a pilot EQA scheme for genetic testing for FRDA, with 10 participants from 10 different European Union countries. No genotyping errors were recorded, but a small number of errors of interpretation were identified. Accordingly, a workshop was organised to develop consensus best practice guidelines for FRDA testing. These guidelines are available at [www.emqn.org](http://www.emqn.org). An open FRDA EQA scheme was offered in 2000. Thirty-four laboratories including 3 from the USA participated. In contrast to the pilot scheme, this open EQA identified deficiencies in the tests in use in several laboratories, and revealed wide variation in the quality of interpretation of results. A clear need for reference samples for accurate sizing was also identified. Feedback from the scheme has been very positive; a further round of EQA will be carried out in 2001.

**The process of developing an educational pamphlet regarding hereditary breast cancer for un- and under-insured women.** *W.F. Cohn*<sup>1</sup>, *D. Brower*<sup>2</sup>, *J. Bauerle*<sup>3</sup>, *S.M. Jones*<sup>4</sup>, *S. Miesfeldt*<sup>3</sup>. 1) Health Evaluation Sci; 2) Curry School of Education; 3) Dept Internal Medicine; 4) Cancer Center; Univ Virginia, Charlottesville, VA.

Although cancer genetics services are increasingly available, access is limited among those who are un- or under-insured. We are developing an educational pamphlet to help un- and under-insured women understand and assess their potential risk for hereditary breast cancer (HBC). The pamphlet has two primary sections: (1) information on topics such as HBC risk and resources and (2) a workbook attachment that allows women to identify characteristics of their personal or family histories that may put them at risk for HBC. We followed an instructional design process to guide the development of the pamphlet. This involved several steps, including: goal development, instructional analysis, and analysis of learners and context. The educational goals of the pamphlet are to enable users to: (1) identify risk factors for HBC; (2) understand the importance of knowing if one is at risk for HBC; (3) determine one's potential risk for HBC; and (4) know available resources for women at risk for HBC. The instructional analysis involved identifying steps and subskills for each instructional goal, so that baseline knowledge needed for goal attainment would become evident and could be included in the pamphlet. The analysis of learners and context involved a small survey of the target population to determine baseline knowledge and learning preferences. Results indicated that most respondents understood the concepts of *hereditary* (19/20), *inherited* (15/20), *hereditary breast cancer* (19/20), and *inherited breast cancer* (15/20). A majority also understood the concepts of *risk* (12/20) and *risk factor* (15/20). Thus, these terms will be included in the pamphlet without extensive definition. Few women were able to define the phrase *early-onset* (4/20). This concept will be explained in the pamphlet. The pamphlet will enable women to self-assess potential risk for HBC and access appropriate resources. Future plans include a formative and summative evaluation of the pamphlet prior to wider distribution.

To be presented by L. P. ten Kate instead of L. Henneman.

Program Nr: 1542 from the 2001 ASHG Annual Meeting

**Preconceptional cystic fibrosis carrier screening: evaluation of a pilot study.** *L. Henneman<sup>1</sup>, L.P. Ten Kate<sup>1</sup>, G. Pals<sup>1</sup>, I. Bramsen<sup>2</sup>, H.M. Van der Ploeg<sup>2</sup>.* 1) Clinical and Human Genetics, VU Medical Center, Amsterdam, the Netherlands; 2) Medical Psychology, VU Medical Center, Amsterdam, the Netherlands.

The feasibility of preconceptional cystic fibrosis (CF) carrier screening in the Netherlands was evaluated using four different ways of inviting and educating couples. Invitations, by mail, were either by general practitioner (GP) or by municipal health services (MHS). Education was either individually at GP's consultation or at a predetermined general educational session. Participants' and non-participants' motives, reasons for (non-)attendance, knowledge, attitudes and sociodemographics were addressed using questionnaires. In total, 38,291 persons (20-35 years) received a first invitation. It turned out that only 20% of them formed part of an eligible couple. Of these, 559 couples consented to test. Participation of couples at the educational session invited by GP or MHS was 12% and 9% respectively. Uptake was higher when couples were educated at GP's consultation (25%). The main reason given for non-participation was lack of time to attend the educational session (53%) or GP's consultation (36%). Overall, other reasons given were that the couples did not want to know their results (28%), lack of concern (6%), and fear for results (5%). Sixty-nine percent of non-participants asserted that screening should be offered routinely to couples planning children. In two approaches, a second invitation was sent, one year after the first, and another 3-7% of eligible persons came forward. GP's did not report any negative experiences. Participating couples provided mouthwashes of both partners. Testing was, however, step-wise. Eighteen carriers were identified; their partners tested negative. Satisfaction among participants was high: 95% would make the same decision to be tested again, and 89% would recommend testing to others. Our results show that the primary care setting provides highest couple attendance, although knowledge of couples educated by the GP was lower than couples educated at the educational session. Furthermore, repetition of the invitation increased the uptake. Overall, these results suggest that preconceptional carrier screening is feasible in the Netherlands.

**Simple fluorescent PCR assay for discriminating FRAXA fully mutated females from normal homozygotes.** *J. Lourdaux<sup>1</sup>, C. Houdayer<sup>1</sup>, T. Billette de Villemeur<sup>2</sup>, G. Royer-Legrain<sup>3</sup>, M. Bahuaud<sup>1</sup>, JP. Bonnefont<sup>3</sup>, D. Feldmann<sup>1</sup>, R. Couderc<sup>1</sup>.* 1) Biochimie et Bio Moléculaire, Hospital Trousseau; 2) NeuroPédiatrie, Hosp. Trousseau; 3) Biochimie Génétique, Hosp. Necker; Paris, France.

Fragile X syndrome is the most common inheritable genetic disease accounting for mental retardation. Despite robustness, Southern blot-based diagnosis is not suitable for large-scale routine screening necessary for neuropediatric practice. PCR appeared as an interesting alternative and various protocols were successfully applied to molecular screening in mentally retarded boys and girls, in whom two alleles with one-repeat difference could be evidenced (CGG repeat heterozygosity among normal females is about 70%). Unfortunately, discriminating the remaining 30% normal homozygote females from full-mutated ones remains a difficult task. Indeed, failure of identification by PCR is accounted for by competition of the wild-type allele with the expanded one during the PCR process. Therefore, we opted for another approach in designing a semiquantitative PCR assay, based on the amplification of the wild type allele. This method allowed us to detect the presence of one or two normal alleles with the same sizes, thereby evidencing a FRAXA fully mutated female or a normal homozygote, respectively. A previously described protocol [Houdayer et al. Clin Chem Lab Med 1999; 37:397-402] was modified by using a limited number of cycles and a reduced amount of enzyme. As an internal control, DNA from a normal boy (with a different repeat number from the female sample studied) was added to the PCR mixture to monitor possible fluctuation in PCR reaction yield. PCR products were electrophoresed using an ABI 373A or 310 (Applied Biosystems). Mutational status was determined by the comparison of fluorescence intensity between peaks generated from the sample and peaks generated from normal or full-mutated female controls. After an optimization step, a preliminary trial on 50 female DNA samples allowed us to ascertain the mutational status in all patients. We believe this simple PCR assay is a powerful approach that would reduce the recourse to Southern-blotting in females with mental retardation of unknown etiology.

**Detection of trinucleotide repeat disease using micro-capillary electrophoresis chip.** *Y-E. Lee<sup>1</sup>, S-J. Baek<sup>1</sup>, H-J. Kim<sup>1</sup>, S-C. Jung<sup>2</sup>, J.K. Jeong<sup>1</sup>.* 1) Microarray Center, Biomedlab Institute, Biomedlab Co., Clinical Research Institute 5304, Seoul National University Hospital, Jongno-Gu, Yongon-Dong 28, Seoul 110-744, Korea; 2) Department of Biomedical Sciences, Division of Genetic Disease Research, Korea National Institute of Health, Eunpyong-Gu, Nokbun-Dong 5, Seoul, 122-701, Korea.

Several major human single gene disorders are attributed to the expansions of highly unstable trinucleotide repeat (TR) sequences. TR numbers are closely related to not only the onset age and severity but also diagnosis and prognosis of disease. Therefore, it is very effective and essential to estimate the accurate TR size for screening or confirmation of trinucleotide repeat disease (TRD). DNA testing such as southern blotting or polymerase chain reaction (PCR) has been commonly used in the diagnosis of TRD. However, such methods require laborious steps for the diagnosis and lack accuracy in detection of carriers and estimation of TR numbers. Therefore, we have developed a new method for the detection of TRD using micro-capillary electrophoresis chip (micro-CE chip). Amplified target sequence by PCR using specific primers designed for TR was separated in micro-capillary electrophoresis (CE) chip, having 15 mm deep, 60 mm width and 35.5 mm length separation channel. TR amplicon was detected by and the size of TR was determined by confocal detection method using the semiconductor diode laser (635 nm). We evaluated the method by analysis of samples from normal subjects, 9 HD patients, 13 SBMA patients, 2 DRPLA patients, 6 FX carriers and 3 DM carriers. Southern blotting method was simultaneously performed to confirm our results. The data obtained from the micro-CE chip and southern blotting were highly comparable. The estimated TR number was also confirmed by sequencing. This study suggests that micro-CE chip is very useful for the detection of TRD and the determination of TR numbers. This new method could be very helpful in early diagnosis, carrier testing, and proper genetic counseling.

**Molecular analysis of FRAXA mutations: advantages limits and diagnostic implications of an improved PCR-amplification method.** *R. Polli, F. Finco, M. Martella, L. Picci, F. Zacchello, A. Murgia.* Dept Pediatrics, Univ Padua, Padua, Italy.

Fragile X molecular testing is aimed at fully characterizing the extent of the FRAXA repeats expansions and the state of methylation of the FMR1 gene promoter and repeated sequences, and at defining the size of gray-zone alleles. Southern blot, which allows the complete analysis of these mutations, is considered the method of choice, even though not accurate in the resolution of small-size alleles and relatively labor-intensive. The attempts to simplify Fragile X analysis by several PCR amplification protocols, have not yielded convincing results, mainly due to the very high CG content and to the size these DNA stretches. A new PCR method that for the first time seems to efficiently amplify FRAXA full mutations has been recently developed and made available. We have been using this tool to retrospectively analyze samples of mutated individuals from 44 Fragile X families previously studied by conventional Southern blotting, to compare advantages and limits of the two different systems and verify the possibility of modifying our current molecular approach to routine FRAXA diagnosis. Preliminary results obtained from the analysis of 40 samples of FRAXA full and pre-mutations show amplification of fragments with a maximum of 700 repeats. It has been possible to identify, in a few samples, the presence of fragments of pre and full mutation that had not been detected by Southern blotting. These latter data could modify the final interpretation of the molecular analysis in those samples and increase the number of the observed mosaic cases. The use of Southern blot is definitely still necessary not only for a complete methylation analysis of the Fragile X region, but also for the reliable detection of extreme repeat expansions. On the other hand, the introduction of a FRAXA-specific PCR amplification system proved to be highly sensitive and efficient can contribute to improving the accuracy of Fragile X molecular testing and can increase our understanding of the molecular characteristics of these complex mutations. Cofinanziamento MURST 2000 MM06195974\_001.

**Screening of the CTG/CAG expansion at the Huntington's Disease-Like 2 locus (HDL2) in patients with Huntington's disease.** *G. Stevanin<sup>1</sup>, C. Julien<sup>1</sup>, S.E. Holmes<sup>2</sup>, C.A. Ross<sup>2</sup>, R.L. Margolis<sup>2</sup>, A. Brice<sup>1</sup>, A. Durr<sup>1</sup>.* 1) INSERM U289, Hopital de la Salpetriere, Paris, France; 2) Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Huntington's disease (HD) is a neurodegenerative disorder resulting primarily from the loss of neurons in the striatum. Chorea is associated with cognitive impairment with a progressive and unremitting course. A CAG repeat expansion in the IT15 gene on chromosome 4 is responsible for the vast majority of cases with HD phenotypes and autosomal dominant transmission of the disease. Recently, a CTG/CAG repeat expansion in a new gene (HDL2 locus) was detected with the Repeat Expansion Detection method in patients from a large HD kindred without expansions in the IT15 gene (Holmes et al, ASHG 2001 abstract).

To verify the involvement of this new repeat expansion in unexplained HD cases and its frequency, we screened 95 controls and 83 patients with HD phenotypes, in whom expansions in the DRPLA and IT15 genes had previously been excluded. The number of repeats varied from 8 to 28 units in the controls. The distribution did not vary with geographical origin and was not significantly different in the patients (10 to 17 repeats). Only one Moroccan patient carried 50 uninterrupted CTG/CAG repeats.

The patient, a 44 year old woman, presents with a 2 years duration of mild choreic movements of the face and extremities associated with reduced Mini Mental Status (22/30). Neuropsychological evaluation revealed sub-cortical dementia similar to that found in HD caused by expansion in the IT15 gene. Enlargement of the anterior horn of the lateral ventricles was observed on cerebral MRI suggesting atrophy of the head of the caudate nucleus. The father died accidentally at age 54. No other affected individual is known in the paternal family.

In conclusion, this observation confirms the genetic heterogeneity of HD, but CTG/CAG repeat expansions at the HDL2 locus account for only 1% of our series of Huntington's disease-like patients without expansions in the IT15 gene.



**FRAXE screening among males with idiopathic mental impairment in Brazil.** *C.B. Santos, M.M.G. Pimentel.*  
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The FRAXE mutation is caused by an unstable expansion of a polymorphic CCG repeat within the 5' untranslated region of the FMR2 gene, located at Xq28, 600kb distal to FRAXA. As a consequence of the excessive number of triplet repeats, a nearby CpG island is hypermethylated and the FMR2 gene is turned-off, resulting in the phenotype features, such as, mental impairment or learning difficulties, speech delay, poor writing skills and hyperactivity. Based on size and methylation status, FRAXE alleles could be divided into four classes: normal (6-30 CCG), intermediate (31-60 CCG), premutated (61-200 CCG) and full mutated (over 200 CCG). In this study, a linear series of 226 special education-needs, developmentally delayed or language delayed male patients, aging from 2 to 18 years old, were screened for the FRAXE expansion. The subjects were previously referred for the Fragile X testing, but found to be negatives for full mutations across the FRAXA *locus*. We have developed a non-isotopic polymerase chain reaction (PCR)-based assay for the identification of FRAXE full mutation alleles among males. Amplification products were resolved by electrophoresis in nondenaturing 6% polyacrilamide gels stained by silver. None of the patients tested positive for the FRAXE expansion, suggesting that FRAXE is not a common aetiological factor among this group of patients. It is tempting to speculate that FRAXE full mutation bearers often escape ascertainment bias, due to the non-specific mild phenotype and the well adaptation to the environment. Therefore, it was postulated recently that the FRAXE full mutation prevalence is higher (1/23.423 males) than the estimated FRAXE prevalence of 1/50.000 males. In conclusion, once molecular analysis of FRAXE mutation is now available, further screening studies are needed for the purpose of identifying newly FRAXE patients and improving genetic counseling.

**Rapid HPLC Purification of Labeled and Unlabeled Oligonucleotides.** *K.R. Yardley<sup>1</sup>, P.D. Rainville<sup>1</sup>, M. Gilar<sup>1</sup>, E. Budman<sup>1</sup>, M. Berne<sup>2</sup>, R.J. Russell II<sup>1</sup>.* 1) Life sciences applications, Waters Corp., Milford, MA; 2) Physiology, Tufts University school of medicine, Boston, MA.

High purity oligonucleotides are essential for many types of genomic assays including detection of single nucleotide polymorphisms (SNPs) and quantitative PCR. Because the purity of synthetic oligonucleotides is typically ~ 70% (for 25-mer), purification is necessary to remove failure sequences and unlabeled target molecules. The current oligonucleotide purification methods include ion-exchange HPLC and PAGE purification, however, these methods suffer in recovery and/or purity of target product. We have developed a rapid, robust HPLC method for analysis and purification of unlabeled and fluorescently labeled oligonucleotides using ion-pair reversed-phase chromatography with the XTerra® 2.5mm MS C18 column. This single step HPLC separation is capable of purifying labeled and unlabeled oligonucleotides from failure sequences and unlabeled products. Results for unlabeled oligonucleotides are exceptional, typically > 95% purity, with >90% recovery. We have also used this method to purify oligonucleotides containing fluorescent labels including Cy5, FITC, TAMRA, and TaqMan™ to > 90% purity with > 85% recovery. This method far exceeds current purification technology in both recovery and purity for labeled and unlabeled oligonucleotides.

**Purification of synthetic oligonucleotides - Utilizing denaturing high performance liquid chromatography (dHPLC).** *S. Sheikhavandi*<sup>1</sup>, *J. Riviello*<sup>2</sup>, *L. Bao*<sup>2</sup>, *J. Adams*<sup>3</sup>, *M. Marino*<sup>4</sup>. 1) Applied Genomics and Molecular Genetics, Transgenomic, Inc., San Diego, CA; 2) Separations Research and Development, Transgenomic Inc., San Diego, CA; 3) Market Development, Transgenomic Inc., San Diego, CA; 4) Applied Genomics and Molecular Genetics, Transgenomic, Inc., Gaithersburg, MD.

The genomics revolution has rapidly increased the demand for highly purified synthetic oligonucleotides for PCR, cloning, genotyping, chip-based technologies, and various applications in the field of pharmacogenetics. Gel electrophoresis and high performance liquid chromatography (HPLC) have previously been the most commonly used techniques for oligo purification. Compared to both gel electrophoresis and typical IP-RP HPLC, dHPLC offers the advantages of higher resolution, larger size range for separation, purification chemistries, reproducibility, automation, and convenient data archiving. We describe the use of dHPLC for the analysis and purification of synthetic oligonucleotides. The unique separation chemistries of the OligoSEP® column combined with an elevated temperature of 70<sup>0</sup>C offer rapid and highly efficient separation and purification. The WAVE® OLIGO system is a fully automated dHPLC system specifically designed for the analysis and purification of synthetic oligonucleotides. Using thermally stable polymeric reversed phase columns in the reversed phase ion- pairing in fully denaturing mode, a broad range of synthetic oligonucleotides can be purified in less than 10 minutes. In addition, by changing the eluent chemistry, separations can be performed based either on oligo length alone, or a combination of length and base pair composition. The purification of unmodified, dye labeled, and biotinylated oligonucleotides have also been accomplished using this technique.

**Genomic approaches for identifying genes involved in auditory hair cell regeneration.** *D. Hawkins<sup>1</sup>, S.*

*Bashiardes<sup>1</sup>, N. Fukushima<sup>1</sup>, N. Saccone<sup>1</sup>, F. Li<sup>1</sup>, G. Stormo<sup>1</sup>, M. Warchol<sup>2</sup>, M. Lovett<sup>1</sup>.* 1) Washington University, St. Louis, MO; 2) Central Institute for the Deaf, St. Louis, MO.

In higher vertebrates sensory hair cell loss leads to permanent deficits in hearing and equilibrium. However, hair cells in the ears of many nonmammalian vertebrates can regenerate after injury. Replacement hair cells are produced by renewed proliferation of epithelial supporting cells. In the avian cochlea these cells are normally quiescent, but will proliferate in response to the death of hair cells. In contrast, supporting cells within the chick utricle are in a constant process of proliferation. We are measuring differences in gene expression between normal cochlea and utricle, and changes that occur in the cochlea during hair cell regeneration, by employing three custom cDNA microarrays. The first array consists of ~400 genes that previous studies have identified as being expressed in the inner ear. The second is an array of 10,000 genes from sequence verified human cDNA clones. The third is an oligonucleotide array of 50mers designed to detect the great majority of transcription factor genes. To derive expression profiles we have employed micro-cDNA synthesis methods to enable us to profile gene expression with less than 50,000 cells. In a comparative analysis of gene expression in the sensory epithelia of proliferative utricles and quiescent cochleas we have detected >4-fold increases in the utricle of the transcripts encoding tyrosinase-related protein, cadherin-4, neuregulin-1, and connexin 46.6, among others. Among the changes in the cochlea are higher levels of expression of the retinoic acid receptor responder 3, neurofibromatosis type 2, and dynein light chain genes relative to utricle. We are also enriching for differentially expressed genes in the utricle compared to the cochlea using a cDNA subtraction method. By applying this method through three rounds of subtraction we have reduced the level of common abundant cDNAs by >100-fold and have enriched for utricle-specific cDNAs. By a combination of these approaches we intend to identify key genes in the process of chick hair cell regeneration and to then explore their regulation in the mammalian inner ear.

**Molecular cloning of a human novel gene MLEL1 of a homologue of Drosophila Mle protein and its tissue expressive profile analysis.** *J. Fu, L. Li, G. Lu.* Laboratory of Human Reproductive Engineering, Central South University, Xiangya Medical College. Changsha, 410078 P. R. China.

The genome of *Drosophila Mle* has been sequenced, which is an ideal model organism for the study of structure genomics and function genomics. The maleless gene (*mle*) with RNA and DNA helicase function in *Drosophila*, is involved in gene expression post-translation regulation in the germline in *Drosophila*. The *mle* gene belongs to superfamily of DEAD/DEAH, and all possess of a conserved motif including Asp-Glu-Ala-Asp/His. The function of these proteins presents not only the splice, but also the cell functions including ribosome assemble, translation initiate, spermatogenesis and embryogenesis. In this paper, applying the strategy of homologue cloning from the beginning of MLE protein in *Drosophila*, the human novel gene, named MLE like 1 (MLEL1) has been cloned by combined with bioinformatic analysis and experience techniques (AF217190). MLEL1 gene have 3600 base pair (bp) long, contained a 3024 bp open reading frame with a longer DNA/RNA helicase box (DEAD/DEAH). The amino acid protein sequences of MLEL1 in human and MLE in *Drosophila* are 38% identical and 57% positive. Northern blotting showed a 3.8 kb single strong signal in testis, and others only very weak 3.8 kb hybridization pattern or not. MLEL1 gene has been mapped on chromosome 3q25.1-3q25.2 by chromosome localization analysis and 26 exons and 25 introns have been confirmed primarily by genomic structure analysis. MLEL1 may be involved in human spermatogenesis and male reproductive.

**Meta-learning for Combining Multiple Classifiers.** *X. Fang*<sup>1</sup>, *M. Xiong*<sup>2</sup>. 1) Statistics, Beijing University, Beijing, P. R. China; 2) Human Genetics Center, University of Texas - Houston.

Emerging advances in microarray technology and gene expression based classification are serving as precursors of whole genome functional analysis and revolutionizing disease diagnosis. There exist many classification methods. However, different classifiers may not only differ in their global performance, but they also may show strong local differences. Each classifier may have its own region in the feature space where it performs the best. To combine multiple classifiers will improve the accuracy of classification. Meta-learning is a technique that seeks to construct higher-level classifier that combine multiple classifiers based on different learning methods or different data sets. Due to the high cost of most microarray experiments, having the ability to utilize multiple data sets will be extremely valuable for biomarker identification and disease classification. In this report, we develop an adaptive boosting algorithm for meta-learning that seeks to compute a "meta-classifier" that integrates the separately learned classifiers to boost overall accuracy of classification. The proposed boosting algorithm will be applied to combine three classifiers based on linear discriminant analysis, logistic regression and support vector machines for classifying colon tumor tissues.

**Collection, storage, and distribution of data concerning DNA variations affecting human phenotypes.** *R.G.H. Cotton<sup>1</sup>, A.D. Auerbach<sup>2</sup>, P. Bork<sup>3</sup>, A. Brookes<sup>4</sup>, A.J. Cuticchia<sup>5</sup>, O. Horaitis<sup>1</sup>, H. Lehväsliho<sup>6</sup>, C.J. Porter<sup>5</sup>, C.R. Scriver<sup>7</sup>, C.C. Talbot Jr.<sup>9</sup>, G.R. Taylor<sup>8</sup>, S.A. Teebi<sup>5</sup> and members of the HUGO Mutation Database Initiative.* 1) Genomic Disorders Research Ctr, St Vincents Hospital Melbourne, Australia; 2) Rockefeller University, New York, U.S.A; 3) European Molecular Biology Laboratories, Heidelberg, Germany; 4) Karolinska Institute, Stockholm, Sweden; 5) Bioinformatics Supercomputing Centre, The Hospital for Sick Children, Toronto, Canada; 6) EMBL Outstation, European Bioinformatics Institute Wellcome Trust Genome Campus, Hinxton, UK; 7) McGill University, Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada; 8) Imperial Cancer Research Fund, Mutation Detection Facility, Leeds, UK; 9) The Johns Hopkins University, Baltimore, USA.

We outline a plan to ensure that variation in genes, particularly mutations causing single gene disorders, is collected from laboratories worldwide, quality controlled, deposited for safe-keeping in a central database, and made readily available to the public. We plan to develop a community ethic in which mutation/variation submission is routine, to encourage and make such submission simple, and to develop a central database and appropriate systems to safeguard such data while making them available to the public and public databases. The collection of all variation (approximately 1/2 of known gene mutations now remain unpublished) will ultimately be important for human health as genomic disease causing alleles eventually affect 60% of the population in a lifetime. The MDI now proposes to develop a system to encourage and ensure variation capture. Receipt and capture of data will be enabled by a "WayStation" with Editorial review assisted by the MDI. A "Warehouse" will be established to store and distribute these data. Novel aspects of this system are its systematic involvement of the community, including worldwide collection of clinical mutations, the sophisticated Waystation entry point, acting as an electronic peer reviewed journal, and the Warehouse to act as the central database. See the MDI Website: [www.genomic.unimelb.edu.au/mdi/](http://www.genomic.unimelb.edu.au/mdi/).

**A comprehensive approach to whole genome SNP analysis and bioinformatics.** *A.M. Brower, D. Baranski, J. Baier, E. Beaty, B. Tremelling, V. Lyamichev, M. De Arruda Indig, M. Atilas, S. Law, B. Neri, R. Ganske, L. Fors.* Bioinformatics, Third Wave Technologies, Madison, WI.

As a result of the Human Genome Project (HGP), over 29 gigabytes of data is currently available in a number of public databases, and over 2.8 million candidate SNPs have been submitted to NCBI's SNP database (dbSNP). Twelve thousand entries describing human genetic disease have been submitted to the Online Mendelian Inheritance in Man database with over 9288 having a gene locus and 1124 of these identified as having allelic variants. It becomes clear that the ability to find and catalog SNPs has surpassed our capacity to validate and genotype these variants, and to describe the resulting genotype/phenotype correlation. We present a comprehensive, interactive sequence analysis pipeline designed to create synergy between sequence information of varying completeness, candidate SNPs, and disease genes. We created several BLASTable databases representing draft and finished sequence, mRNA sequences, and SNP sequences. We then developed a suite of computational tools and an *in silico* sequence analysis pipeline to mine and automatically harvest information to annotate the genomic landscape surrounding each SNP for 100 kilobases, resulting in a comprehensive and interactive database of SNPs grouped by their functional characteristics. The resultant SNP profiles include information relating to GC content, allele frequencies, population statistics, map location, amino acid change, protein motifs, and functional location (exonic/intronic). The SNPs can be easily grouped into blocks to create single multi-allelic loci for use in linkage disequilibrium studies as microsatellite replacements for gene mapping studies. In addition, SNPs in coding, splice sites, and regulatory regions of genes can be used in candidate gene studies and for fine mapping efforts. The SNPs are being used to populate panels of Invader assays targeted to address specific questions related to disease gene discovery, disease susceptibility, diagnosis and treatment. We will present a genome wide map of SNPs which incorporates allele frequencies from our genotyping of four distinct populations.



**Exclusion of SINEs from imprinted regions dates from the earliest mammalian lineages.** *J.M. Greally*. Department of Medicine (Hematology), Albert Einstein College of Medicine, Bronx, NY.

To test the hypothesis that regions undergoing genomic imprinting have unique genomic characteristics, a number of major parameters of sequence heterogeneity in the human genome were identified and used to compare imprinted with non-imprinted loci. Imprinted regions were found to be normally permissive to retroposition events in general, as judged by the similarity in frequency of recent L1 insertions in imprinted and non-imprinted regions. However, imprinted regions were found to have a universal and significant decrease in short interspersed elements (SINEs: both Alu and MIR elements). Paternally- and maternally-expressed imprinted genes were separately compared with the control sequence panel, each showing the same marked decrease in SINE content. Ongoing integration of SINEs occurs within the imprinted regions, as the distribution of Alu subclasses of different evolutionary ages is similar in imprinted and control loci. Further findings were that maternally- and paternally-expressed imprinted genes differed in terms of their GC, CpG and L1 content, indicating that they may segregate into separate genomic compartments, and that CpG islands were larger at promoters of imprinted than non-imprinted genes. Active selection against a level of SINE retroposition above a critical threshold may occur due to the property of certain SINEs to attract and spread methylation, which could cause functionally deleterious consequences in imprinted regions already undergoing methylation and silencing of one allele. As MIR elements are common to all mammals, including marsupials and monotremes, the paucity of MIRs in imprinted regions indicates that imprinting or the genomic conditions allowing it preceded the evolution of placental mammals. The low frequency of SINEs suggests that a function of imprinted regions may be to act as sentinels against high levels of potentially mutagenic transposon activity in the developing organism, by providing large, dispersed genomic targets in each germline that are critical for embryonic development and are sensitive to the presence of newly-retroposed and methylated SINEs.

**Automated Quality Analysis and Functional Annotation of Public SNP data.** *N.W. Brown<sup>1</sup>, J.J. DiStefano<sup>1</sup>, S.F. Nelson<sup>2</sup>.* 1) Computer Science, UCLA, Los Angeles, CA; 2) Human Genetics, UCLA, Los Angeles, CA.

The practical use of public genomic data requires assessing its accuracy, its integration with other genomic data and the qualitative and quantitative predictions of the functional implications of these data. This often requires the integration of data from many sources. This integration is problematic in that public genomics data sources are: 1) very often not well organized 2) of varying error rates 3) referenced/cross-referenced between or within the data source in poor or non-existent manner and 4) devoid of functional implications. Automatic approaches to retrieve, quality check, cross-reference and predict the functional consequence of these data is crucial to the practical use of genomic information. Here we present a scheme for the automated quality analysis and functional annotation of polymorphism data from dbSNP. Our approach uses heuristic rules and probabilistic models to integrate and predict the confidence of the existence of a single nucleotide polymorphism, its allele frequency and the functional severity of allelic differences from public SNP data.

**Parallelization of GENIGMA, a novel analytical tool for large-scale gene expression microarray studies.** *D. Bozinov, C.M. Burson, O. Spiegelstein.* Ctr Human Molecular Genetics, Univ Nebraska Medical Ctr, Omaha, NE.

Microarray experiments with two tissue samples labeled respectively with Cy-3 and Cy-5 can instantly produce datasets of tens of thousands of expression values. For computational purposes this data size seems still reasonable on modern computer workstations. However, many scientifically interesting questions can only be answered by analyzing multiple microarrays of this size. Such an extensive scientific study creates a multidimensional matrix with hundreds of thousands of data points. For such a vast amount of expression data sophisticated computational tools are critical in order to extract meaningful information. GENIGMA, a novel software tool for high-throughput analysis and interpretation of gene expression microarray data has been developed to manage and process up to one thousand arrays at a time. Various statistical methods are in place to offer a sophisticated means for advanced analysis of either the entire study or only a subset of arrays. Unfortunately existing algorithms for clustering characteristically show a time complexity of quadratic polynomial expansion. Therefore, the effective processing time increases dramatically for large data sets. New strategies for comprehensive gene clustering on massively parallel processor (MPP) systems are discussed, which result in substantial performance gain. Extensions for parallel processing of gene expression data as well as structural modifications of GENIGMA are described. The recent improvements of this software tool make it exceptionally powerful and well prepared for upcoming large-scale microarray studies.

**A Gene Expression Profile of Aging in Mice.** *Z. Chen, B.L Merriman, S.F. Nelson.* Human Genetics, UCLA, Los Angeles, CA.

The genetic basis of aging in mammals is poorly understood. At this early stage, it is desirable to take a broad, unbiased look at what is actually occurring at the genetic level during aging. DNA microarrays are a new technology that enable us to monitor gene expression in such a broad fashion. We apply this technology to a mouse model in order to directly look at age-related changes in mammalian gene expression on a genomic scale. Our approach is to measure gene expression levels in mice representing a variety of ages, from young adult to elderly, and to look for gene expression changes correlated with age. We monitor expression in a variety of tissues, to assess tissue-specific variations. The mice used were male BALB/c, at ages of 5, 8, 12, 17, 22, 25 months, and a pair of mice at each age level was used for replication. From each mouse we harvested multiple organs (brain, liver, kidney, muscle and heart) and expression in each was monitored separately. We use spotted cDNA microarrays containing 8600 mouse genes to measure gene expression in the tissue samples, and hybridizations were done in comparative fashion, using the young adult (5 month old) tissues as reference samples. Thus a total of 50 arrays were required for this study. We applied clustering methods to the resulting expression data to identify groups of genes with similar expression changes during aging, and similar behavior across multiple tissues. In this initial survey of 8600 genes, approximately 200 showed a clear up-regulation trend with age, while 100 showed a clear down regulation trend. Within these major groups are subgroups with more similar trends, including several "aging marker genes" that show dramatic change (up to 32 fold) at extreme ages, as well as subgroups containing genes connected to energy metabolism, biosynthesis, cell cycle regulation, and stress response. Overall the results suggest that the normal aging process in mammals has a moderate impact on the expression of hundreds of genes involved in many basic cell processes, while very few genes show striking alteration in expression levels.

**High throughput SNP analysis and drug target prioritization.** *J.P. Alsobrook II<sup>1</sup>, C. Burgess<sup>1</sup>, M. Grosse<sup>1</sup>, D. Lepley<sup>1</sup>, E. Szekeres<sup>2</sup>, L. Shien<sup>2</sup>, J. Bader<sup>2</sup>, R. Shimkets<sup>1</sup>.* 1) Internal Discovery, CuraGen Corp, New Haven, CT; 2) Bioinformatics, CuraGen Corp, New Haven, CT.

Genes differentially expressed between normal and diseased states offer attractive targets for drug-based intervention. CuraGens proprietary gene expression technologies (SNPCalling<sup>TM</sup>, SeqCalling<sup>TM</sup>, GeneCalling<sup>TM</sup>, PathCalling<sup>TM</sup>) employ an integrated bioinformatics platform for the rapid identification of potential human disease targets and pharmacogenetic markers. The GeneCalling<sup>TM</sup> and SNPCalling<sup>TM</sup> processes, respectively, compare the gene expression and polymorphism profiles of disease or drug-response models to appropriate controls. Identification of differentially expressed genes does not require prior knowledge of gene sequence, a direct contrast to conventional chip-based technologies. Downstream polymorphism analysis of these potential targets is supported by advanced automation and informatics technologies, as well as our proprietary human sequence database (SeqCalling<sup>TM</sup>) consisting of more than 4 million sequences. Target selection is further refined through the integration of the targets gene expression and polymorphism profiles with our extensive protein:protein interaction database (PathCalling<sup>TM</sup>) and high-throughput tissue distribution characterization. A complete profile of the selected target can be rapidly engineered, dramatically reducing the non-productive consumption of resources while increasing the efficiency of selection. Further laboratory validation efforts are thereby directed toward targets that have the highest potential for successful downstream drug development or therapeutic intervention.

**Post sequencing gene discovery; novel genes in human chromosome 21 (HC21).** *S. Deutsch*<sup>1</sup>, *A. Reymond*<sup>1</sup>, *R. Lyle*<sup>1</sup>, *S. Antonarakis*<sup>1</sup>, *A. Camargo*<sup>2</sup>, *S. de Souza*<sup>2</sup>, *A. Simpson*<sup>2</sup>, *B. Stevenson*<sup>3</sup>, *C. Iseli*<sup>3</sup>, *P. Bucher*<sup>4</sup>, *V. Jongeneel*<sup>3</sup>. 1) Group A: Division of Medical Genetics, University of Geneva Medical School, Switzerland; 2) Group B: Ludwig Institute for Cancer Research, Sao Paulo, Brazil; 3) Group C: Ludwig Institute for Cancer Research, Epalinges, Switzerland; 4) Swiss Bioinformatics Institute, Epalinges, Switzerland.

The initial annotation of the complete sequence of HC21 revealed 151 genes and 79 predicted transcripts identified by gene prediction programs and the presence of spliced ESTs (Nature 405: 311, 2000). However, the gene identification strategy pursued for chromosomes 21 and 22, and for the rest of the genome is probably biased against genes with small ORFs, and/or large 5 and 3 UTR. In our effort to refine HC21 sequence annotation, we have re-analyzed the entire chromosome sequence incorporating new ESTs (including the ORESTES database), and a new algorithm for stringently mapping 3 polyadenylation tags. 3 tags were extracted using raw chromatogram data from EST projects, and were quality controlled by considering multiple factors including polyadenylation signals, position of polyA in the sequence, and presence of artifactual genomic priming sites. These 3tags represent unambiguous and reliable markers for transcripts. We built an HC21 ACE database in order to visualize all potential transcripts together with the 3 tags, CpG islands, repeats and protein domains all of which contribute to the identification of putative genes. We thus defined a restrictive set of criteria that allowed the identification of 27 previously undescribed candidate transcripts, which are being experimentally confirmed by full EST insert sequencing, 5 and 3 RACE, and RT-PCR. More than a third of these putative transcripts have already been verified, and one maps within a previous sequencing gap of HC21. The revised HC21 transcriptome will have implications for the rest of the genome concerning the quality of previous annotations and the total number of transcripts. It will also provide new candidates for genes involved in Down Syndrome and other genetic disorders that map to HC21.

**Multiple Comparison Analysis Program: further analysis of GeneChip data.** *Y.V. Kotliarov<sup>1</sup>, S.E. Kotliarova<sup>2</sup>, N. Nukina<sup>2</sup>.* 1) Research into Artifacts, Center for Engineering, University of Tokyo Tokyo, Japan; 2) Lab for CAG Repeat Diseases; Brain Science Institute, RIKEN, Japan.

Transcriptome analysis using microarrays became a powerful tool in various areas of genetics and molecular biology. Usually researchers face the problem of multiple comparisons of several control data with several experimental data (e.g. several control animals versus several transgenic animals). The task is to fish out really affected genes (e.g. differentially expressed in transgenics vs controls) among the noise data (due to individual variation or experimental error). There is lack of software which allows to fulfil this task at present. We developed the program Multiple Comparison Analysis (MCA), which makes possible such analysis and, using the power of comparison analysis of multiple pairs, extracts additional information from the comparison data provided by Data Mining Tool and Microarray Suite softwares (Affymetrix).

MCA is written using VBA on MS Excel-2000 and therefore can run on both Windows and Macintosh computers. Briefly, the software groups the data from several pair-wise comparisons into one class by the same probe set, counts the members in the class, checks the presence of signal in all pairs, checks consistency of differential expression among the members of the same class. In addition, it averages several parameters (such as average difference, fold change, etc) in a class and, finally, makes decision of overall change in pairs of the same class. MCA, combined with the advanced features of MS Excel (Microsoft), brings additional power to microarray analysis using GeneChip (Affymetrix) to mine efficiently important information from the huge amount of biological data. The software was tested and used in practical analysis by researchers of RIKEN and of the University of Tokyo.

**Annotation of the Human Genome: Use of the Genome as an Organizing Principle for Data Integration.** *N.*

*Litchfield*<sup>1</sup>, *N. Tsinoremas*<sup>2</sup>. 1) Marketing & Communications, DoubleTwist, Inc, Oakland, CA; 2) Computational Genomics Group, DoubleTwist, Inc, Oakland, CA.

Analysis of publicly available human genomic sequences has yielded a range of gene predictions sorted by type and depth of evidence. As there is no single accepted algorithmic methodology to identify human genes, we have created an analysis pipeline based upon a combination of ab initio gene finders and mapping of additional experimental evidence onto the genome. This pipeline is executed in a parallel mode to optimize our ability to predict human genes. The first line of experimental evidence used in this study was clustered human and mouse expressed DNA sequences (ESTs). Mapping this information onto the genome provides very strong indication of the existence of genes when such information is available. The second line of experimental evidence includes mapping of all known proteins that share significant similarity with human genomic sequences. This powerful approach allows us to identify genes based on protein similarities where the other methods fail to provide evidence of a gene. Furthermore, we synthesize all this information using a rule-based system that combines each type of evidence and generates reports with varying confidence levels with respect to the existence of genes in a particular position on the genome.



**"SNP-Cruncher": A Perl Script Toolbox for Large-Scale SNP Data Mining and its application on 13q32.** *C. Liu, E.S. Gershon.* Dept Psychiatry, Knapp Res Ctr, Univ Chicago, Chicago, IL.

As the Human Genome Project comes to its final stages, more than 93% of the draft human genome sequence has been deposited into public databases, and millions of candidate SNPs (Single Nucleotide Polymorphisms) have been discovered. This data is essential for association analyses of complex disorders. In order to explore approaches required for a small lab to analyze all available genome data related to a region, we targeted the SNPs in chromosome 13q32 where a Bipolar Disorder susceptibility locus has been reported. "SNP-Cruncher" consists of 65 Perl programs, integrated by hyperlinks in a Microsoft PowerPoint file (or, alternatively, in a web page). Two categories of programs are provided. One group is standalone tools, providing functions including SNP restriction enzyme analysis, primer evaluation, and homology-based SNP mapping/annotation. Another group is "bridge tools," providing the input data preparation and output data interpretation for existing genomic analysis programs, as well as database contents preparation and update. With these "bridge tools," publicly available software (such as BLAST, RepeatMasker, Phrap, Primer3, Sim4, Vector NTI, and DNASTAR, etc.) can be used to analyze thousands of sequences easily. We analyzed 6349 SNPs on 13q32 from NCBI dbSNP. 21% of the SNPs were found to be duplicated or closely mapped. 40% of the SNPs were found to be suitable for PCR-RFLP analyses with inexpensive enzymes (one of the Perl programs of SNP-Cruncher). 57.5% of the SNPs analyzed contain more than 60% repeat sequences, and consequently may be unsuitable for use. 3.6% of the SNPs (226 SNPs) matched transcript sequences, and therefore may be potential cSNPs. A local database storing data for further systematic marker selection is generated as part of the analyses, and can carry annotation forward when new genomic or expressed sequence becomes available.

**Molecular dissection of the contribution of single genes to changes in global gene expression in Down syndrome.**

*R. Lyle<sup>1</sup>, R. Chrast<sup>1</sup>, C. Gehrig<sup>1</sup>, P. Chanson<sup>1</sup>, C. Hendrich<sup>1</sup>, H. Scott<sup>2</sup>, S. Antonarakis<sup>1</sup>.* 1) Department of Medical Genetics, Centre Medicaire Universitaire, Geneva, Switzerland; 2) Walter and Eliza Hall Institute, Melbourne, Australia.

Down syndrome (DS) or Trisomy 21 is the most common genetic cause of mental retardation, occurring in approximately 1/700 live births. In addition, there are other neurological phenotypes associated with DS, including brachycephaly, microcephaly and early onset Alzheimer's like disease. The presence of an extra copy of certain but not all of the approximately 250 genes on HC21 is likely to contribute to the DS phenotype. HC21 is largely syntenic with a region of mouse chromosome 16 and thus a partial trisomy 16, Ts65Dn, acts as a model of DS and shares many of the DS phenotypes. We have used Serial Analysis of Gene Expression (SAGE) and filter microarrays containing ~25,000 mouse genes and ESTs to analyse changes in gene expression in whole brains of Ts65Dn mice. Both analyses revealed complex changes in gene expression. Several lines of evidence suggest that dysregulation of the signal transduction cascades of Sim2 and Dyrk (Mnbh), a transcription factor and kinase respectively, are likely to be involved in development of DS phenotypes. To help dissect the contribution of the Sim2 and Dyrk to the Ts65Dn expression pattern, we have overexpressed their cDNAs in cell culture using adenoviral infection and analysed induced changes in gene expression using mouse filter arrays. A combination of these in vivo (Ts65Dn) and in vitro (Sim2 and Dyrk adenoviruses) data compared to controls should allow us to begin to decipher the molecular cascades leading from gene triplication to the complex DS phenotype.

**Molecular characterization of the human fovea using gene expression profiling and bioinformatic analyses.** *P.S. Lagali<sup>1</sup>, B.A. Wilton<sup>1</sup>, G.D. Lutzak<sup>1</sup>, D.K. Patterson<sup>1</sup>, K.R. Robson<sup>1</sup>, S.L. Bernstein<sup>2</sup>, P.W. Wong<sup>1</sup>*. 1) Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada; 2) Department of Ophthalmology, University of Maryland, Baltimore, MD.

The human fovea is a small region of the retina containing the highest density of cone photoreceptor cells and is responsible for mediating fine visual acuity. Many inherited retinal disorders involve the dysfunction or degeneration of cells comprising the fovea, particularly diseases of the macula, which represent the most common cause of blindness in the developed world. To gain insight into the normal biology of the fovea, and to uncover potential contributing factors in the progression of retinopathies, several approaches were taken. A primary human fovea cDNA library was differentially screened to identify fovea-expressed genes that are associated with a particular tissue distribution, species specificity, and temporal expression pattern. Such analysis identified a number of genes characterized by neural tissue-enriched expression, sequence conservation across diverse species, or exhibiting age-related changes in expression level. These in turn represent genes that may be critical for fovea function and that are also possible candidate genes for retinal disorders affecting the foveal region. In addition, a macroarray containing 18,394 unique human ESTs was screened with human fovea total cDNA in order to identify fovea-expressed genes. 500 of the ESTs representing high to moderate abundance transcripts were further analyzed to determine their cytogenetic locations, thus enabling the creation of a primary transcript map of the human fovea. The genetic map locations of several of the corresponding putative genes coincide with chromosomal intervals to which retinal diseases have been mapped, thereby identifying potential candidate genes for these disorders. The methods utilized in this investigation have enabled molecular characterization of an understudied ocular tissue and have provided tools for the identification of possible disease genes that play a role in its dysfunction. [Supported by CIHR, AHFMR, the E.A. Baker Foundation of the CNIB, Foundation Fighting Blindness-Canada and NSERC].

**Enhanced Performance of SeqScape™ Software Version 1.1 for Comparative DNA Sequencing Analysis and**

**Mutation Detection.** *C. Kosman*<sup>1</sup>, *G. Chappell*<sup>2</sup>, *J. Sorenson*<sup>2</sup>, *Q. Doan*<sup>2</sup>, *P. Suri*<sup>2</sup>, *R. Paul*<sup>2</sup>, *B. Kshirsargar*<sup>2</sup>, *A. Sauve*<sup>2</sup>, *G. Mason*<sup>2</sup>, *P. Honebein*<sup>4</sup>, *G. Denisov*<sup>5</sup>, *M. Schoppe*<sup>3</sup>, *A. Pronayev*<sup>3</sup>, *V. Bawge*<sup>4</sup>, *L. Johnston-Dow*<sup>2</sup>. 1) Genetic Analysis, Applied Biosystems, Foster City, CA; 2) Informatics, Applied Biosystems, Foster City, CA; 3) Group Logic, Arlington, VA; 4) Systems Integration Solutions, San Francisco, CA; 5) Paracel, A Celera Company, Pasadena, CA.

SeqScape™ software is a Windows NT®- and 2000®-compatible program containing completely integrated base calling, sequence assembly, alignment and comparison tools which allow for the rapid and accurate analysis, and alignment of multiple sample sequences containing mixed-base positions against a reference sequence. Version 1.1 of this software contains several important feature enhancements to increase the quality of the analysis results and decrease user intervention. Specifically, the accuracy of base calling is increased through the use of TraceTuner™ Version 2.0 which supplies superior mixed-base calling and per-base confidence values. While the accuracy of consensus generation is significantly improved through the use of a novel comparative sequencing consensus caller with per-base confidence values and new algorithms for data trimming. Results from the performance evaluation of this new software tool on several challenging comparative sequencing data sets are shown. For these analyses, this new application produced superior results while providing substantial performance advantages over other similar assembly programs tested. In all cases, the innovative base calling and sequence assembly features included in SeqScape™ software Version 1.1 resulted in improved base calling and assembly accuracy, less manual data manipulation and shorter turnaround times for the complete analysis of comparative sequencing projects.

**Improved Quantitation of Affymetrix GeneChip Microarray Data.** *B.L. Merriman, Z. Chen, S. Nelson.* Human Genetics, UCLA, Los Angeles, CA.

The Affymetrix GeneChip is a commercially available DNA microarray system for large scale gene expression analysis in human, mouse and other organisms. The technology is based on the ability to synthesize short oligo DNA probes (25-mers) at high density (400,000 distinct probe spots on a 1.3 cm. square) on a glass substrate. In the GeneChip system, each gene being monitored is represented by many oligo probes---typically 32-40 probes per gene, with half of these being 25-mer sequences from the transcript, and the other half obtained by introducing a single base change into each of these sequences. The analysis software reduces these 40 hybridization signals per gene into a single measurement of gene expression level, the "Average Difference", which is the average of the differences in hybridization signal between the match and mismatch probes. There is little published data that validates this approach to producing a single gene expression number from the 40 measurements available. Moreover, in 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. For example, 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. Also, the Average Difference measurement of gene expression typically does not show as large a range of variation as more direct experimental measurement techniques, such as SAGE or EST counting. This suggests that there may be more accurate ways of extracting gene expression information from the underlying oligo hybridization signals provided by the GeneChip System. We investigate this possibility in detail, based on the analysis of over 100 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 also compare these quantitation methods with SAGE and EST counting results. By creating a customized .CEL data file, these alternative gene expression measures can be input into the Affymetrix Analysis Suite software for data mining purposes.

**A complete mutation analysis panel of the 39 human HOX genes.** *K. Kosaki<sup>1</sup>, R. Kosaki<sup>2</sup>, K. Sasaki<sup>1</sup>, H. Yoshihashi<sup>1</sup>, M. Tomita<sup>3</sup>, N. Matsuo<sup>1</sup>.* 1) Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan; 2) Department of Medical Genetics, Saitama Children's Medical Center, Iwatsuki, Japan; 3) Laboratory for Bioinformatics, Keio University, Fujisawa, Japan.

The HOX gene family consists of highly conserved transcription factors that specify the identity of the body segments along the anteroposterior axis of the embryo. Because the phenotypes of mice with targeted disruptions of Hox genes resemble some patterns of human malformations, mutations in HOX genes have been expected to be associated with a significant number of human malformations. Thus far, however, mutations have been documented in only 3 of the 39 human HOX genes (HOXD13, HOXA13, and HOXA11). In the present study we took advantage of the human and mouse draft genome sequences to determine the genome structure of 17 human HOX genes. When neither human nor mouse full coding cDNA sequences were available in the GenBank database, the phylogenetic footprints of the HOX clusters were delineated by comparison of the human genome sequence containing the HOX cluster with the corresponding mouse genome. In addition, the genomic region with coding potential was selected by probabilistic prediction method of the coding sequences. Based on this sequence information, we successfully developed a set of PCR primers to amplify the entire coding region of each of the 39 HOX genes from genomic DNA in 125 amplicons. Our results indicate the usefulness of bioinformatic analysis of the draft genome sequences for clinically oriented research projects. It is hoped that the mutation panel provided here will serve as a launchpad for a new discourse on the genetic basis of human malformations.

**An Independent Evaluation of the Human Genome Sequence.** *J.R. Lupski, K.C. Worley, N. Katsanis.* Baylor College of Medicine, Houston, TX, USA.

In June 2000, the completion of a draft version of the human genome comprised of multiple short contigs which encompass 85% or more of euchromatin was announced and the detailed findings of the sequencing consortium were reported several months later. The new information will allow global questions to be addressed, such as total gene numbers and a more accurate definition of gene families. Of equal importance, however, are positional questions such as local genome architecture, regional gene density, and location of transcribed units, the latter being critical for disease gene evaluation. We have performed a series of mapping and computational experiments using a non-redundant collection of 925 ESTs and the draft genome sequence available at different time points between April 2000 and May 2001. By comparing our EST mapping information obtained from two independent physical mapping methods to that obtained from sequence matches to draft genomic sequence, we estimated the percent of EST clones that could be mapped, and the ability to use genome information to map accurately any given EST. We have found substantial discrepancies in both the coverage of the human genome and the accuracy of mapping of genomic clones which suggests some limitations of the draft human genome sequence in providing positional information and detailed characterization of chromosomal subregions.

Program Nr: 1570 from the 2001 ASHG Annual Meeting

**Mouse Genome Database: Curation Strategies for Alleles and Phenotypes in the Mouse.** *C.L. Smith, J.A. Blake, J.E. Richardson, J.A. Kadin, J.T. Eppig and the Mouse Genome Informatics Group.* Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME. (<http://www.informatics.jax.org/>).

The Alleles and Phenotypes section of The Mouse Genome Database (MGD) seeks to represent mouse mutants and models of human genetic disorders and associated phenotypic characteristics in a robust yet easily searchable format. In addition, MGD provides integrated access to extensively curated data on the genetics, comparative genomics and mapping of these mouse mutants as well as gene expression data from the Gene Expression Database (GXD) and tumor biology from the Mouse Tumor Database (MTB).

A major effort is underway to create a structured, controlled vocabulary to describe phenotypic abnormalities in the mouse. In addition to facilitating computational data curation and mining, this set of terms will expedite curation of and queries for phenotypic information in MGD. The terms will also enhance integration between phenotypes and other relevant information, such as genes, expression data, and comparative maps. Furthermore, the vocabularies will be structured to allow complex queries involving multiple characteristics or expression and mapping data that are difficult to retrieve by plain text searches. Standard vocabularies are especially important due to the rapid production of phenotypic mutants generated at the distributed ENU mutagenesis centers.

A further refinement in allele curation is the adoption of standardized allele and transgene nomenclature in curated data sets, providing databases and investigators a mechanism to name and differentiate similar mutations. Allele nomenclature guidelines are set by the International Committee on Standardized Genetic Nomenclature for Mice.

MGD is supported by NIH/NHGRI grant HG00330.



**A Tabu Search Algorithm for Gene Selection in Whole Genome Functional Analysis.** *M. Sun*<sup>1</sup>, *M. Xiong*<sup>2</sup>. 1) Business, University of Texas at San Ant, San Antonio, TX; 2) Human Genetics Center, University of Texas - Houston, Houston, TX 77225.

Completion of the human genome sequence marks a switch from a genome sequence era to an age of functional genomics and will have a profound impact on biomedical research. Currently, the major tools for mapping disease genes are based on meiotic mapping within the paradigm of positional cloning. A road less traveled is functional analysis. How to systematically and efficiently identify disease-associated genes and biomarkers of drug action using whole genome gene expression profiles obtained from DNA chip has not been fully explored. In this report, we will propose to use classification as a general framework and to develop feature wrappers and filters as major statistical methods for gene selection. Since whole genome gene expression profiles, in general, involves thousands or even ten thousands of genes, both feature wrappers and filters, incurs the high computational cost of conducting a search through the huge space of feature subsets. To develop efficient algorithms to search subsets of genes with the optimal criterion values is essential to the success of disease-associated genes and biomarker discovery. In this talk, we will present a novel search algorithm for feature selection which is computationally less expensive and is able to search global optimal subsets of genes and biomarkers. Unlike traditional feature selection algorithms which search only an optimal subset, the search algorithms in our biomarker and gene identification problem should be adapted to finding all possible optimal subsets of genes.

**The Generalized T2 test for Biomarker Identification Using Gene Expression Data.** *J. Zhao, M. Xiong.* Human Genetics Ctr, Univ Texas Houston, Houston, TX.

The identification of specific targets for diagnostic or therapeutic use has been a principle goal for both clinicians and biomedical researches. So far, in biomedical research, major methods for identification of disease genes is based on positional cloning, and linkage and linkage disequilibrium analysis. Alternative approach to the discovery of disease genes and biomarkers for diagnosis of disease and measuring of drug efficacy and toxicity is whole genome functional analysis, which incorporates feature selection into gene expression pattern recognition. However, pattern recognition methods, in general, do not provide p-values to evaluate the significant evidence for biomarker identification. In this report, we propose a generalized T2 test and a sequence forward floating search algorithm for biomarker discovery. The proposed method not only can identify single biomarker, but also can identify a composite biomarker which consists of several genes. In this report, we will also reveal a close connection of the proposed T2 test statistic with the discriminant analysis. The proposed method will be applied to the real gene expression data sets.

**In Silico Cloning: the identification of genes within the CLN6 critical region.** *R.B. Wheeler<sup>1</sup>, J.D. Sharp<sup>1</sup>, R.A. Schultz<sup>2</sup>, S.E. Mole<sup>1</sup>, R.E. Williams<sup>1</sup>, R.M. Gardiner<sup>1</sup>.* 1) Dept Paediatrics, RFUCMS, The Rayne Institute, London, England; 2) Eugene McDermott Center for Human Growth and Development, University of Texas SouthWestern Medical Center, Texas, USA.

CLN6, the gene for variant late infantile neuronal ceroid lipofuscinosis, maps to a 1cM region on chromosome 15q22-23 between microsatellite markers D15S988 and D15S1000. A physical map of the region has been constructed and two PAC clones from the region have been fully sequenced at the University of Texas. A total of 316,633 base pairs of sequence have been deposited at <http://gestec.swmed.edu>. A set of overlapping BAC clones which span the entire 1 Mb CLN6 critical region have been identified by virtual STS content mapping using NIX (<http://menu.hgmp.mrc.ac.uk/Nix>) and the Human Genome Browser (<http://genome.ucsc.edu>). Using this data we identified additional simple repeats in silico using the program Tandem Repeat Finder (<http://c3.biomath.mssm.edu/trf.html>). Marker typing in informative families enabled us to reduce the critical region from 14 to only 9 BACs. Gene prediction and homology searches were performed on all sequences in the critical region and to date 10 genes have been identified. Of these 7 transcripts have homology to known genes and 3 represent novel transcripts. The coding sequences of 7 of these genes have been fully sequenced in a subset of the CLN6 patient resource and to date no mutations have been identified. We are using a variety of methods to identify further genes within the region including NIX gene predictions, Unigene homologies and ORF predictions from the Human Draft Browser Sequence. This has allowed us to identify a number of potential genes and we are currently testing these predictions in the laboratory.

**Microarray analysis of changes in skeletal muscle gene expression in response to insulin.** *G.P. Page<sup>1</sup>, V. So<sup>2</sup>, J. Ding<sup>2</sup>, L. Mainous<sup>1</sup>, J. Rosinski<sup>2</sup>, B. Rhees<sup>2</sup>, M. Martin<sup>2</sup>, W.T. Garvey<sup>1</sup>.* 1) Medical Univ. South Carolina, Charleston, SC; 2) Dept. Genomic and Info. Sci., Hoffmann-La Roche, Inc., NJ USA.

Insulin is one of the most important and powerful hormones in the human body. The binding of insulin to the insulin receptor causes a cascade of reactions including protein phosphorylation through the PI-3-kinase pathway and de novo transcription through the ras transcription activation complex. We have analyzed skeletal muscle biopsies from 55 individuals pre and post euglycaemic insulin clamp using the Affymetrix Hu95A-E microarrays to look for alterations in gene expression. We have identified 1009 genes (at  $p < 0.05$ ) as being differentially expressed in response to insulin many of which have interconnected functions. For example, in polyamine metabolism SLC7A5 (+1.8 Standard deviations) and SLC7A6 (+0.7 SD) transport arginine into the cell. ARG1(+0.57 SD) metabolizes arginine into ornithine and urea. ODC1 (+1.1 SD) converts ornithine into putrescine. AMD1 (+1.4 SD) metabolizes s-adenosylmethionine into decarboxylated s-adenosylmethionine, which is the primary substrate for converting putrescine into spermidine and spermidine into spermine. ODC1 degradation is inhibited by Antizyme inhibitor (+0.9 SD). The combined increased expression of SLC7A5, SLC7A6, ARG1, ODC1, AMD1, and antizyme inhibitor would be expected to greatly increase the intracellular levels of polyamines which are known to affect DNA methylation levels. S-adenosylmethionine also will donate a methyl group during DNA methylation via DNMT1 (+0.4 SD) and becomes S-adenosylhomocysteine, which may be metabolized into homocysteine and adenosine by AHCYL1 (+1.3 SD). Elevated homocysteine levels are common in type 2 diabetics and are a risk factor for cardiovascular disease. AHCYL1 expression is significantly elevated in insulin sensitive, insulin resistant, and type 2 diabetic individuals in response to insulin. The high levels of insulin seen in diabetics and insulin resistant individuals may induce the elevated plasma homocysteine levels seen in these conditions. Using DNA microarrays with proper experimental design can provide important insights into the pathophysiology of insulin action and diabetes.

**Developing an automated tool for characterizing EST sequences from a human cartilage-specific cDNA library.**

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Despite the huge number of EST sequences in the public domain, it is very likely that there are still some genes found in rare tissues that are under-represented. In order to find and characterize cartilage-specific genes, a pilot study was performed. A previously-generated human cartilage cDNA library derived from fetal 16 week to 2 year costochondral cartilage was prescreened for clones known to be abundantly expressed in this tissue. Five hundred "negative" clones were sequenced by the Human Genome Sequencing Center at Baylor College of Medicine and the output was used to develop an automated tool for identifying novel genes, which are specific for or predominantly expressed in cartilage. A limitation of single pass sequencing is that the sequence quality is highly variable and the reads may be error prone. The best available model for assessment of sequence quality comes from the program PHRED. A 2 pole Tschebysheff low pass filter was employed to improve precision and predictability of identification of the high quality region. This enabled simplified processing of the quality scores and assessment of high quality regions and lead to demonstrably more efficient detection of overlap sequences in EST data sets. Based on this trimming a search pipeline against public databases (mainly NCBI) was developed to divide the cartilage EST sequences into different groups. One group contains all ESTs which match already known and characterized genes. In another one group all ESTs are sorted to match sequences in the public EST databases, but whose gene identity is unknown. The last and most interesting group of ESTs do not match sequences in the public domain and may represent putative novel and/or cartilage-specific genes. In order to minimize false positives in the last group of ESTs, we have studied and optimized the effects of differing search parameters in each of the search steps.

**An equivalence test for comparing DNA sequences.** *H. Thijs<sup>1</sup>, K. Van Steen<sup>1</sup>, G. Molenberghs<sup>1</sup>, M. De Wit<sup>2</sup>, M. Peeters<sup>2</sup>*. 1) Center for Statistics, Limburgs Universitair Centrum, Diepenbeek, Belgium; 2) Tibotec-Virco, Mechelen, Belgium.

All genetic instructions of an organism are stored in code in its DNA. It is a linear molecule that can be seen as a necklace of simple building stones, called nucleotides and represented by the letters A (adenine), C (cytosine), T (thymine) and G (guanine). Recently lots of effort is put in the comparison between sequences of DNA and more specifically one is interested in testing equivalence between two or more sequences by means of statistical methodology. These statistical methods were first developed in the context of pharmacokinetics and later extended to the field of clinical trials (e.g., Dunnett and Gent, 1977). Nowadays, (bio)-equivalence tests are well-known in the drugs testing field, but less frequently used in other fields. Mc Bride (1999) claims that there appear to be few reported applications of equivalence tests in environmental science and management. We feel that also in statistical genetics, testing of interval hypotheses via equivalence test procedures needs a push forward.

Two different DNA sequences can be considered as ratings on several characteristics (loci). From this perspective, we are interested in the closeness or similarity of ratings as a measure of agreement. Closely linked to the concepts of similarity of ratings is the idea that the level of disagreement between any two ratings might be represented by some sort of distance measure. Here, distance should be seen as a measure in an abstract space that has the same mathematical properties as that used for the more familiar 3-dimensional physical world (Dunn, 1989). We therefore propose a Mahalanobis-type index which enables us to recognize a certain degree of uncertainty in the measurements. This uncertainty may be caused by missing observations, inaccurate measurements or ambiguity. After defining this new distance measure we will construct a proper testing procedure through simulation studies which validates the new procedure.

Program Nr: 1577 from the 2001 ASHG Annual Meeting

**Nomenclature: The language of Genomics.** *H.M. Wain, E.A. Bruford, R.C. Lovering, M. Lush, M.W. Wright, M.S. Povey.* HUGO Gene Nomenclature Committee, The Galton Laboratory, Department of Biology, University College London Wolfson House, 4, Stephenson Way London NW1 2HE.

"One gene, one name" is an important concept for accurate communication in genetic research. The HUGO Gene Nomenclature Committee (HGNC) has so far been responsible for naming one third of the genes estimated from the draft of the human genome sequence. With around 20,000 more genes to name we are investigating new initiatives that will give the greatest benefit to the scientific community, specifically recognising that flexibility is required and that names are no substitute for functional annotation. Recent discoveries have increased our understanding of the variety of functional molecules which can be generated from the human genome. Therefore, in line with recent requests, we propose to update our guidelines and policies, increasing the range of genomic features named. These include families of genes with constant or variable regions, which generate alternative proteins depending on their splicing assembly e.g. protocadherins. Better ways are also needed to identify untranslated mRNAs, antisense RNAs, ribosomal RNAs and genes only found within subsets of the population (usually formed by different duplication events). We are also considering nomenclature for other structures in the genome such as LINEs, SINEs and transposable elements. Decisions will be made on an individual basis, so that we can provide a nomenclature which fits the need researchers have to discuss these features. A first draft of the new guidelines will be found on the HUGO Gene Nomenclature Committee webpage at URL <http://www.gene.ucl.ac.uk/nomenclature/> from October 1st 2001. They will be discussed at the pre-ASHG Nomenclature workshop and made available at the ASHG meeting. We can be contacted by email at [nome@galton.ucl.ac.uk](mailto:nome@galton.ucl.ac.uk).

**A comprehensive online human genomic catalog.** *P.S. White<sup>1</sup>, E.P. Sulman<sup>1</sup>, E.P. Katz<sup>1</sup>, S. Sabuktagin<sup>2</sup>, C.J. Porter<sup>3</sup>, T.C. Matisse<sup>2</sup>.* 1) Dept Pediatrics, Children's Hosp Philadelphia, Philadelphia, PA; 2) Department of Genetics, Rutgers University, Piscataway, NJ; 3) Genome Database, Toronto, ON, Canada.

The identification of loci contributing to complex disease remains a central challenge of biomedical genetics. Upon assignment of a candidate disease locus to a specific chromosomal region, determination of the causal gene currently requires extensive bioinformatics and data mining skills, as available genomic and functional genomic datasets are often poorly integrated. To address this, we have developed a genomic data integration method and resource (eGenome) that comprehensively combines publicly available physical, genetic linkage, sequence, and functional genomic-based data sets into a unified data structure accessible via straightforward Internet queries. Utilizing a relational database, eGenome incorporates data sets from a large number of diverse sources, including GenBank, RHdb, the CEPH Genotype database, dbSNP, UniGene, LocusLink, cytogenetic data from the Genome Database, and large-insert clone data. These data were localized to specific chromosomal locations by three mechanisms: 1) construction of high-resolution, high-confidence radiation hybrid maps; 2) construction of all-inclusive genetic linkage maps; and 3) identification of exact sequence coordinates in human draft and finished sequence contigs. Genomic elements representing genes, markers, and polymorphisms were then normalized to remove redundancies and to identify and annotate discrepancies and errors in localizations. The eGenome process was applied to each human chromosome, resulting in placement and annotation of over 2.5 million elements. This resource has been made available to the general public through a website that provides keyword and positional queries using marker names, cytogenetic band positions, and sequence position. In addition to positional information, eGenome provides element-specific links to a large number of online databases and utilities, including Ensembl, UCSC/Golden Path, GenBank, OMIM, and BLAST. In this way, eGenome serves as a primary portal and data mining entry point for all position-oriented human genetic and genomic research. Access eGenome at <http://genome.chop.edu>.



**Automated classification of SNP genotyping results.** *J. Studebaker<sup>1</sup>, S. Alfisi<sup>1</sup>, W. Ankener<sup>1</sup>, D. Morris<sup>2</sup>, M. Phillips<sup>1</sup>.*  
1) Orchid BioSciences, Princeton, NJ; 2) GeneScreen, Inc., Dayton, Ohio.

High throughput, cost effective, and accurate SNP genotyping is a key to the study of diversity in genomes. We have created a prototype program that reduces the time consuming, costly effort required for human review of the genotyping data. We have used this program on 1.2 million individual assays run over a four-month period using the SNPIT™ single base extension technology on SNPstream 25K systems.

The final step in analyzing results from SNPIT genotyping is human review of a scatter plot with the results for multiple samples and a single SNP. The reviewer checks for low signals in samples, high standard deviations in the clusters (two homozygous and one heterozygous) and other features of the plot. The end result of the review is a grade of Pass or Fail for the results presented in the plot. To increase reviewing throughput, we developed a program that compiles statistics for each assay and classifies it on the basis of the statistics.

Our program groups the scatterplots into classes in a hierarchical method. Some classifications, like PCR failure, reflect operational characteristics, and others reflect characteristics of the SNPs themselves. We chose three classifications as automatic Fails, because the reviewers marked 100% of the results in these classifications as Fail. Accordingly, the program now automatically marks results in these classifications as Fail.

Major benefits from the program have been:

- 1) Elimination of the need to review plots in the classifications automatically marked as Fail
- 2) Presentation of all available plots in a particular classification consecutively, an approach that has proven to speed review

Our next goals are automatic grading of 90% of the plots and extension of the program to other systems that use the SNPIT genotyping technology.

**Analysis and validation of cDNA microarray expression data.** *D.N. Stivers<sup>1</sup>, H.G. Sung<sup>1</sup>, M.D. Story<sup>2</sup>, T. Burrows<sup>2</sup>.*  
1) Dept Biostatistics, U.T. M.D. Anderson Cancer Ctr, Houston, TX; 2) Experimental Radiation Onc, U.T. M.D. Anderson Cancer Ctr, Houston, TX.

The quality of data produced by cDNA microarray assays of gene expression can be uncertain. Issues include differentiating true signal from background noise, error whose variance varies non-monotonically as a function of signal level, and estimating the significance of differences in expression level. We describe a set of microarray experiments and statistical analyses testing the ability of microarray technology to resolve true differences between expression levels without finding spurious difference. We estimate background level parametrically, and combine this with non-parametric estimation of error in signal measurement in order to sort differences in expression levels according to their statistical significance. This includes the case when a gene is found to be "off" (i.e., below threshold) in some experiments, but "on" in others.

**The BiSC WayStation: A Centralized Structure for the Collection of Genetic Variations.** *S.A. Teebi<sup>1</sup>, C.J. Porter<sup>1</sup>, C.C. Talbot Jr<sup>2</sup>, A.J. Cuticchia<sup>1</sup>*. 1) Bioinformatics Supercomputing Centre, The Hospital for Sick Children, Toronto, ON, Canada; 2) The Johns Hopkins University School of Medicine, Baltimore, MD, USA.

The BiSC WayStation is a web-accessible centralized structure for the submission, peer-review, publication and release of genetic variation data. Backed by the HUGO-Mutation Database Initiative, it provides a consistent, flexible interface through which researchers anywhere in the world can report novel variations (both disease-causing mutations and silent polymorphisms such as SNPs) in any gene. Submission comprises a series of streamlined forms that closely follow MDI's recommendations for the content and quality control of variation reporting. Data relating to the variations source (reference), genomic context, DNA and quality are required for submission. Other, optional sections gather data on RNA, protein, populations, clinical features, and database cross-links. CGI scripts for verification of submission integrity are embedded into the submission forms and act as a first rudimentary validation step. Definitive validation is provided by the WayStation Review Network. This structure is made up of locus-specific database (LSDB) curators and orphan gene editors for genes not associated with an existing LSDB. Each member of the Review Board is appointed by the MDI to review submitted variations in a specific gene; they do so via a Reviewer module on the website. Coordinated by MDI, recruitment of a complete Review Board has begun and is ongoing.

It is our intention to make submission via the WayStation equivalent to peer-reviewed publication. To this end, we are currently in discussion with the journal *Human Mutation* and the National Library of Medicine to issue *Human Mutation* IDs and PubMed IDs to WayStation submissions. If implemented, these IDs would act as a record and proof of a valid submission, and, significantly, as an incentive to submit to the WayStation. After validation, the WayStation releases published submissions to the appropriate LSDB, HGBASE, and other databases such as dbSNP and OMIM. A demonstration version of the WayStation may be accessed at <http://www.centralmutations.org/>.

**Characterization of Alu distribution in the working draft sequence of the human genome.** *J.E. Stenger, H. Xu.*  
Center for Human Genetics, Duke University Medical Center, Durham, NC.

We have characterized all Alu pairs on each of the human chromosomes using the December 12, 2000 freeze of the human genome draft sequence at UCSC as the data source. Alu data was incorporated into a MySQL database. By querying our database, we identified inverted Alu pairs that were closely-spaced (<20 bp), full length (> and shared high sequence identity as experiments in a yeast model system demonstrated that such Alu pairs were unstable and thus could be a source of genomic instability in humans. Of the 523, 772 Alus pairs in the human genome that were separated by less than 650 bp, 243 were potentially unstable; many of which were intergenic and thus had the potential to cause Alu mediated rearrangements that could result in genetic disease. We also looked at the relationship between Alu content and gene content. Chromosome 19 had by far the greatest number of Alus per bp (756 vs. 195 Alus per bp in the genome). Although chromosome 19 has 2.8 times the gene density of the average chromosome and 3.9 times the number of alu pairs averaged over the genome, there was not a strict correlation between Alu content and gene density. Chromosomes 19, 7 and 16, respectively, had the greatest density of Alus per gene content of the chromosome, while chromosome 7 has less genes per Mb than the average. When we looked at the frequency of particular groups of subfamilies in GC rich regions we observed that the FLA\* subfamily was least represented among regions containing more than 46% GC content, followed by the Y\* subfamily, the J\* subfamily, and the S\* subfamily, while uncharacterized Alus were the most represented. The order was inverted when the GC content fell beneath 38% GC. Interestingly, among individual subfamilies, Alu-Sbcg was by far the most abundant in regions containing greater than 54% GC content.

**Integration of rat, mouse and human EST and comparative maps.** *T.E. Scheetz<sup>1</sup>, M.R. Raymond<sup>2</sup>, A. McClain<sup>2</sup>, M.B. Soares<sup>3</sup>, T.L. Casavant<sup>1</sup>, V.C. Sheffield<sup>2</sup>.* 1) Electrical and Computer Engineering, Univ Iowa, Iowa City, IA; 2) Pediatrics and HHMI, Univ Iowa, Iowa City, IA; 3) Physiology, Univ Iowa, Iowa City, IA.

We have created a set of integrated EST and comparative maps for the human, mouse and rat. These maps consist of almost 60,000 radiation hybrid mapped genes and ESTs across all three species. Specifically, they consist of over 14,000 RH-mapped rat ESTs generated locally at The University of Iowa (1), 12,000 RH-mapped mouse ESTs available from the mouse RH map site at MIT (2), and more than 30,000 mapped human ESTs and genes from GeneMap99 (3). This mapping data was used to identify syntenic segments between each pair of species separately, as described in (4). At least two elements from each species must co-localize before a syntenic segment is defined.

The results of this comparative analysis were then integrated into a comprehensive database with a dynamic, web-accessible interface that is publicly accessible via <http://ratEST.uiowa.edu/>. This resource is expected to be useful in better utilizing the available animal models of human diseases.

Planned extensions to this research include integration of disease interval annotation, and the integration of genomic sequence for all three species as it becomes available.

1. [http://www-genome.wi.mit.edu/mouse\\_rh/](http://www-genome.wi.mit.edu/mouse_rh/)
2. <http://ratEST.uiowa.edu/>
3. Deloukas, P., et al., *Science* 282, 744-746, 1998.
4. Scheetz, T.E., et al., *Genome Research* 11, 497-502, 2001.

**Integration of draft sequence data into the Genome Database (GDB).** *W. Zhu<sup>1</sup>, C.C. Talbot Jr.<sup>2</sup>, C.J. Porter<sup>1</sup>, K. Li<sup>1</sup>, S. Murthy<sup>1</sup>, A.J. Cuticchia<sup>1</sup>.* 1) Bioinformatics Supercomputing Centre, The Hospital for Sick Children, Toronto, ON, Canada; 2) Genome Database, The Johns Hopkins University School of Medicine, Baltimore MD, USA.

For the past ten years, the Genome Database (GDB) has presented human genome mapping data to the research community. During this period, as the resolution of the available maps has increased, GDB moved from providing only cytogenetic localizations to displaying linkage, radiation hybrid and contig maps. Further developments allowed us to display our own integrated comprehensive map, and more recently sequence feature maps that position markers on the evolving draft sequence contigs by electronic PCR.

We are now working to integrate positional data from the draft sequence more closely with the mapping data already in GDB. We have created a draft sequence database that stores the positions of GDB objects on the sequences of the Golden Path and NCBI contigs. A browser application allows the contigs to be searched for specific markers, and extensive links between the browser and GDB allow rapid retrieval of additional information. This allows the user to move easily and rapidly from the sequence data to GDB's extensive curated information. The contigs can also be searched with positional queries, or browsed on a chromosome-by-chromosome basis.

When moving from GDB to the sequence browser, or searching the browser for a particular marker, the user can choose to compare positions within the two contig assemblies currently stored. The database makes it possible for further assemblies to be displayed alongside.

The integration of this new resource into GDB provides a site where researchers can compare mapping results based on sequence, and on more traditional mapping methods. We are investigating ways of displaying these comparisons to researchers in a meaningful way.

**The skeletal genome anatomy project (SGAP).** *T.M. Teslovich<sup>1</sup>, D.P. McKeane<sup>1</sup>, K.E. Pearson<sup>1</sup>, C. Francomano<sup>2</sup>, R. Hotchkiss<sup>3</sup>, D.A. Stephan<sup>1</sup>.* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, D.C. 20010; 2) National Institute of Aging, NIH, Bethesda, MD 20892; 3) OrthoGene, Inc. and the Hospital for Special Surgery, NY, NY 10021.

Diseases pertaining to skeletal tissue affect millions of Americans. Most notable is osteoporosis, which afflicts 8 million women and 2 million men in the United States; an additional 18 million Americans have low bone mass and are at risk of developing the disease. Osteoporosis is responsible for more than 1.5 million fractures annually, including 300,000 hip fractures. Within one year of hip fracture, up to 20% of victims will die, 25% of survivors will be confined to long-term care facilities, and 50% will experience long-term loss of mobility. Osteoporosis-related health care costs are estimated at \$14 billion annually, and osteoporosis-related injuries significantly decrease the quality of life for millions of people. While factors such as diet, exercise, and smoking have great influence, research at the National Institutes of Health indicates that genetics may determine up to 80% of a person's risk for osteoporosis. To facilitate the identification of genes involved in osteoporosis and other skeletal diseases, cDNA libraries have been constructed for the purpose of generating full-length coding sequence for skeletal genes. The RNA source for the libraries is fracture-related tissue undergoing healing, which presumably contains most skeletal cell lineages and will allow us to capture the majority of expressed genes. We have clustered 3' reads, identified clones containing full-length inserts via 5' sequencing and informatics, and these unique clusters will be used to construct a custom Affymetrix expression array. The array will be used as a template to screen clinical disease samples. It is hypothesized that novel, highly-differentially regulated genes will be identified which predispose to osteoporosis. In addition, our information platform will be applied to understand why younger patients heal bones faster than older patients and to gain insight into other skeletal disease processes for which there is a known genetic component. The end goal is the design of diagnostics and therapeutics.

**Development of a microarray-based assay for imprinting in the CNS.** *V.L. Buettner<sup>1</sup>, M.E. Barish<sup>2</sup>, J.R. Mann<sup>1</sup>, J. Singer-Sam<sup>1</sup>*. 1) Division of Biology, Beckman Research Institute, Duarte, CA; 2) Division of Neuroscience, Beckman Research Institute, Duarte, CA.

Although imprinted genes play a role in CNS development, and certain genetic disorders of the CNS show a pattern of inheritance demonstrating that imprinted genes are involved, there is no effective screening method for imprinted gene expression. We are therefore focusing on the establishment of a high-throughput assay that would allow the discovery of novel imprinted genes as well as the interrogation of multiple genes in different regions of the CNS and at various developmental stages. The method relies on crosses of mouse strains carrying balanced translocations of chromosomal regions of interest. The resulting progeny include a subset carrying only maternal or paternal copies of the chromosomal regions; the gene expression profile of these two sets of progeny can then be compared by use of high density DNA microarrays. As a model system, we are analyzing RNA from neonates carrying only maternal or paternal copies of proximal chromosome 7, including the chromosomal region corresponding to the human Prader-Willi/Angelman Syndrome domain. To establish the validity of the assay and optimize conditions, we have designed a custom oligonucleotide microarray. In addition to probes for housekeeping genes and known imprinted genes expressed in the CNS, the microarray contains 300 probes for other genes relevant to CNS function, including ~40 that map to proximal chromosome 7. We have thus far demonstrated the reproducibility of the method, and confirmed imprinting of two genes expressed in cerebellum, *Ndn* and *Snrpn*. To establish the limits of sensitivity of the technique, we are currently using the custom microarray to analyze imprinted gene expression in different regions of neonatal brain, and comparing the results with those obtained by quantitative RT-PCR.



**Identification of novel AAA genes as candidate genes for neurologic disorders.** *P. Hedera<sup>1</sup>, X. Zhao<sup>1</sup>, J.K. Fink<sup>1,2</sup>.*  
1) University of Michigan, Department of Neurology, Ann Arbor, MI; 2) Geriatric Research Education Clinical Center, Ann Arbor Veterans Affairs Medical Center.

AAA (ATPases Associated with various cellular Activities) is a diverse superfamily of proteins characterized by the presence of highly conserved AAA motif, including Walker homology sequences and ATP binding consensus. AAA proteins have been shown to play a role in different functions, including protein degradation, organelle biogenesis, vesicle mediated protein transport and regulation of the cell cycle. Recently, two novel AAA genes have been shown to cause autosomal recessive (AR) hereditary spastic paraplegia (HSP) linked to chromosome 16q24.3 (paraplegin) and autosomal dominant (AD) HSP linked to chromosome 2p22-p21 (spastin). Homology between these two new AAA-family members was limited to the AAA domain. At least 11 additional genes have been mapped as a cause of AR or AD HSP. Phenotype of HSP is typically stereotypical with spastic weakness of lower extremities. We hypothesized that genes causing other types of HSP may also belong to the AAA family. To test our hypothesis, we designed degenerated PCR primers based on the conserved 120 amino acids motif containing the ATP binding consensus domain to systematically clone and characterize genes of the AAA family from the human brain. cDNA from both adult and fetal brain cDNA libraries were first amplified with vector primers and used for amplification with the degenerated primers. PCR products of expected size (about 320 bp) were cloned and analyzed. We analyzed a total of 649 clones by hybridization and DNA sequence analysis and identified several known AAA genes, which include proteasome 26 and delta subunits, peroxisomal biogenesis factor 6, spastin and paraplegin-like protein AFG3. Known genes accounted for 90% of all clones. We also identified 25 unique DNA inserts representing novel AAA genes. None of these novel genes mapped to identified HSP loci. These novel AAA genes, expressed in the fetal and adult brain, may be potential candidates for unidentified HSP loci and other neurological disorders.

**Gene Expression Mapping of Various Regions of the Adult Human Brain.** *M.A. Vinores, M.H. Polymeropoulos, C. Lavedan.* Pharmacogenetics, Novartis Pharma Corp, Gaithersburg, MD.

Despite recent advances in Neurogenetics, and in particular the discovery of genes involved in common neurodegenerative disorders such as Alzheimer's and Parkinson's disease, our understanding of the genetic influence and the molecular mechanism of neurological disorders is still limited. The purpose of this study was to determine gene expression profiles of various regions of the adult human brain and to identify genes specifically expressed in these regions. This information would be helpful in better understanding neuronal molecular pathways and the etiology of various neurological disorders and to aid in designing new therapies. Six different regions from the adult human brain were analyzed: cerebellum, thalamus, corpus callosum, caudate nucleus, hippocampus and amygdala. The samples were hybridized to the Affymetrix U95A array containing approximately 12,000 probe sets. Gene expression profile of these various brain samples were compared to each other as well as to a variety of different human tissues. Comparisons of profiles were performed using the in-house Novartis Pharmacogenetics Network (NPGN) database. Gene expression profiles were also analyzed using GeneSpring 4.0.1a software to identify clusters of co-regulated genes within each of the six brain regions. Approximately 300 genes were found to be expressed at a significantly higher level in the human brain than in a variety of other normal human tissues. The number of genes expressed specifically in a single brain region did not exceed 40. These findings provide a global atlas of gene expression in distinct regions of the brain. This gives a new insight into the molecular mechanisms implicated in brain functions, as well as candidate genes that may be involved in common diseases such as Alzheimer's disease, schizophrenia or other psychiatric disorders.

**Expression profiling of the cardiovascular system by the microarray technology.** C.-H. Yi<sup>1</sup>, M. Schinke<sup>1</sup>, J.-H. Kim<sup>2</sup>, P. Jay<sup>1</sup>, T. Shioi<sup>1</sup>, M. Wripple<sup>2</sup>, A. Butte<sup>2</sup>, L. Riggi<sup>1</sup>, D.I.-B. Chen<sup>1</sup>, I.S. Kohane<sup>2</sup>, S. Izumo<sup>1</sup>. 1) Cardiovascular Div, BIDMC, Harvard Medical School, Boston, MA 02215; 2) Children's Hospital Informatics Program, Harvard Medical School, Boston, MA 02215.

We have initiated applying the DNA microarray technology to the expression profiling of the cardiovascular system in various developmental, physiological and pathological states. The gene expression levels of the mouse hearts have been measured by cDNA microarray (Incyte) and oligonucleotide array (Affymetrix). For more reliable signal intensity values and gene identities, various low-level analyses and corrections of raw data from microarray hybridization have been performed. Based upon these refinements, the transcriptomal analysis that utilizes two-point and multiple-point analyses both in combinatorial or novel ways was schematized and performed for the framework of data analysis and interpretation, which are informative of global cellular states or changes, and are harmoniously connected to the follow-up studies with the hypotheses generated. The dataset from the heart undergoing transition from embryonic stage to adulthood and that of a mouse dilated cardiomyopathy model induced by a dominant negative H-Ras in the heart were arbitrarily chosen. The mouse heart undergoing the transition showed a comparatively large transcriptomal alteration, confirming the initial prediction that the cell-division related genes, for example *Cdc2*, *Cdc28*, *Pcna*, be significantly down-regulated. The negative H-Ras mouse model showed a comparatively small change both in gene numbers and in the extent of expression changes, revealing the up-regulation of conventional hypertrophy marker genes, *ANF* and *BNP*. Many new genes that have never been characterized and uncharacterized, up to now, in the cardiovascular system showed significant changes in expression levels. *Cbfa1*, a transcription factor characterized in osteogenesis, was implicated both in the late heart development and the dilated cardiomyopathy. The transcriptomal analysis also revealed that interferon gamma signaling is likely to play important roles both in the cardiac development and in the dilation/hypertrophy process of the negative H-Ras mouse model.

**Identification of noise factors in expression profiling.** *M.A. Bakay, R. Borup, Y. Chen, E.P. Hoffman.* Research Center for Genetic Medicine, Children's National Medical Center, Washington DC.

Expression profiling is increasingly utilized as a genome-wide approach for defining the downstream effects of biological variables on gene expression. We investigated Duchenne dystrophy by comparing expression profiles for 2 groups of DMD patient: 5-6 and 10-12 year old to define the source of variability (experimental, intra-patient (tissue), inter-patient (SNP noise) and disease progression). The analysis group was 34 MuscleChip profiles from 8 controls and 10 dystrophin-deficient patients (5 patients in each age group). Each MuscleChip array contained 4,601 probe sets corresponding to 3,369 distinct genes and ESTs expressed in human muscle. Variables tested included 2 different regions of the same biopsy on duplicate MuscleChip (20 profiles), and mixed patient hybridization cocktails (10 profiles). Unsupervised hierarchical clustering of the 34 profiles showed that the greatest source of variability often originated from different regions of the same patient muscle biopsy. This suggests tissue heterogeneity is a major confounding variable in interpretation of expression profiling data. Mixed patient samples showed the highest concordance of duplicate profiles, showing that tissue heterogeneity and SNP noise variability could be normalized by experimental design. We used a two-sample t-test in GeneSpring software to analyze individual profiles and specify differentially expressed genes that could distinguish between group 5-6 and 10-12 year old DMD patients. We also used our four-fold iterative survival method to obtain fold changes for mixed profiles. Both methods showed patient age was not a predictable variable in expression profiles, despite the fact that this was a progressive disease. In conclusion, we showed by using the custom skeletal muscle-specific GeneChip that variables effecting expression profiles were: tissue heterogeneity>>inter-patient variability>>age of patient. Furthermore, our data showed mixing of patient samples prior to profiling is a valid method for normalizing both inter- and intra-patient variation when studying disorders of known primary etiology preserving most significant and specific gene expression changes.

**Global Analysis of Gene Expression in Inherited Human Craniosinostosis.** *T.S. Zorick<sup>1</sup>, A.Y. Fernandes<sup>4</sup>, P. Turma<sup>2</sup>, H. Matsushita<sup>3</sup>, S. Zanini<sup>5</sup>, A. Palhares<sup>6</sup>, M.R. Passos-Bueno<sup>1</sup>.* 1) Biology, University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil; 2) Plastic Surgery, Hospital das Clinicas, Sao Paulo, Brazil; 3) Neurosurgery, University of Sao Paulo, Sao Paulo, Brazil; 4) Neurosurgery, USP-Bauru, Sao Paulo, Brazil; 5) Craniofacial Surgery, USP-Bauru, Sao Paulo, Brazil; 6) Plastic Surgery, USP-Botucatu, Sao Paulo, Brazil.

Inherited human craniosynostoses are one of the most common inherited human birth defects, occurring in about 1 in every 2500 births. Recently, many craniosynostotic syndromes have had their causative mutations mapped to genes for Fibroblast Growth Factor Receptor (FGFRs) 1-3. However, despite extensive knowledge about the primary genetic changes responsible for the conditions, considerable overlap and clinical variability exists with regards to clinical outcome and phenotypic severity even among individuals possessing identical mutations. We are using fibroblasts cultured from surgical specimens taken during normally scheduled corrective surgeries to perform the SAGE technique in order to understand the pattern of gene expression among the various conditions. We have found some preliminary differences in gene expression between normal human fibroblastic cultures and cultures taken from a patient with nonsyndromic craniosynostosis. We hope to extend this study to other samples taken from patients with identifiable mutations and typical syndromic clinical conditions in the future.

**Evidence for interchromosomal recombination errors as a factor in the duplications of chromosome 15q.** *D-H. Liu, S. Mann, N. Wang, N. Dorrani, C. Schanen.* Dept. Human Genetics, UCLA, Los Angeles, CA 90095.

A common cytogenetic abnormality associated with autism is a supernumerary chromosome derived from chromosome 15, idic(15). Both idic(15) and interstitial duplications [int dup(15)] within 15q11-q13 are thought to arise because of nonhomologous meiotic recombination, usually involving a series of repeated sequences on proximal 15q. In order to analyze the mechanism of formation of these duplications, we genotyped 20 families with duplications of proximal chromosome 15q. Using 9 microsatellite markers flanking the critical region 15q11-q13 (from proximal marker D15S541 to distal marker D15S1031), we found all the duplications were of maternal origin. Among 20 cases, twelve clearly resulted from interchromosomal exchanges, with three distinct alleles from proximal down to distal region. These findings suggest that similar to the deletion events found in Prader-Willi patients, the duplication events often arise due to unequal meiotic exchanges between nonsister chromatids in the maternal meiosis I. Thus, duplications that lead to trisomy for the involved region may involve either maternal heterodisomy or isodisomy, depending upon the origin of the normal maternal chromosome 15. Since the correlation between phenotypic features resulting from duplications appears more complex than simply the amount of involved chromatin, it will be interesting to determine whether different duplication mechanisms might link a clustering of clinical features in AD with a variety of proximal 15q anomalies. Supported by HD 35470.

**Detection of Trisomy 21 using Single Nucleotide Polymorphisms.** *G.A. Pont-Kingdon<sup>1</sup>, E. Lyon<sup>1, 2</sup>*. 1) ARUP Laboratories, Salt Lake City, UT; 2) Department of Pathology, University of Utah.

Numerous SNPs have been mapped along chromosome 21. Using a panel of these SNPs, we have developed a quantitative molecular assay with the LightCycler and hybridization probes for the detection of trisomy 21. This approach should allow fine molecular detection of partial chromosomal duplication. The use of heterozygous SNPs provides an internal control in the assay. Derivative melting curves differentiate two alleles of a given SNP by their different thermodynamic stability with a fluorescently labeled hybridization probe. The temperature ( $T_m$ ) at which the probe denatures from the template and fluorescence signal is lost, identifies the alleles. Hybridization probes were selected to provide a difference in  $T_m$  between both alleles of 7 to 10°C. Alleles are quantified by analyzing the relative area of derivative melting curves. The relative area in non-trisomy 21, heterozygous at a SNP locus should reveal two curves of near equal area, while in heterozygous trisomy 21 samples, one peak should be twice the other. Several SNPs, heterozygous in at least 30% of a random population were selected to provide a test that includes at least two heterozygous SNPs per individual in a large percent of the population. Additional SNPs, in the region believed to be critical for the syndrome are currently being investigated. A consistent difference in peak area ratio between non-trisomy 21 and trisomy 21 samples was observed and indicates the feasibility of the approach. After normalizing to a heterozygous control, non-trisomic samples had a melting curves ratio around 1.0 while trisomy 21 samples had ratios of 1.8 or 0.6 depending on which allele was duplicated. This study opens application of melting curve analysis and can be extended to other chromosomal dosage defects.

**Is a novel fragile site on chromosome 18q22.1 associated with in vivo chromosome breakage? B.MH.**

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At the moment, approximately 30 fragile sites have been described. The majority of them is expressed when cells are grown in folic acid deficient medium, whereas others are induced by the addition of BrdU or distamycin A to the culture medium. After induction of cultured cells with these specific chemical agents, fragile sites appear as non-staining regions, chromatid gaps or sometimes even breaks on metaphase spreads. Only a single fragile site has been observed that also causes in vivo chromosome breakage: FRA11B. Apart from FRA11B, there has been no direct evidence of chromosomal breakage occurring in vivo in carriers of fragile sites. Yet, recently, a novel fragile site was postulated on chromosome 18q22.1 of the father of an infant with an apparent 18q22.1 deletion. In addition, the child has features of Beckwith-Wiedemann syndrome and biallelic IGF2 expression [Brewer et al., 1998]. The fact that the putative fragile site in the father was found on the same location as the chromosome deletion breakpoint in the infant, suggests that the deletion in the infant is associated with the inheritance of the paternal fragile site expressing chromosome 18 and its subsequent in vivo breakage. The current contribution aims at presenting our results on the testing of a possible association between this fragile site and the chromosome deletion in the infant by identifying the chromosome breakpoint and cloning the 18q22.1 fragile site. As a first step, we confirmed the presence of this novel fragile site and showed that the infant has actually a terminal deletion, not due to a translocation. The candidate region of the breakpoint was refined to minimum size. Genes in the region were identified and the region was searched for expanded repeats. The finding that apart from FRA11B also another fragile site is known to cause in vivo chromosome breakage, would make it obvious to implicate fragile sites in a common mechanism of chromosome breaks. Brewer CM et al. 1998. *J Med Genet* 35: 162-164.



**Multiple pathogenic and benign rearrangements arise from an ancient 35-kb genomic duplication involving the *NEMO* and *LAGE2* genes.** S. Aradhya<sup>1</sup>, T. Yamagata<sup>2</sup>, T. Bardaro<sup>3</sup>, P. Galgoczy<sup>4</sup>, T. Esposito<sup>3</sup>, S. Kenwrick<sup>5</sup>, M. Platzer<sup>4</sup>, M. D'Urso<sup>3</sup>, D.L. Nelson<sup>1</sup>. 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, USA; 2) Jichi Medical School, Tochigi, Japan; 3) Int'l Institute of Genetics and Biophysics, Naples, Italy; 4) Institute of Molecular Biotechnology, Jena, Germany; 5) University of Cambridge, Cambridge, UK.

Incontinentia pigmenti (IP) is an X-linked dominant, male-lethal disorder caused by mutations in *NEMO* (*IKK-g*). We recently showed that most IP patients carry the same genomic deletion, due to rearrangement between two identical repeats. Analysis of the IP deletion in male abortuses had suggested the existence of a second, incomplete copy of *NEMO*. Clones containing this truncated copy (*DNEMO*) have now been identified and incorporated into a BAC contig in distal Xq28. *DNEMO* lies in an inverse orientation telomeric to *NEMO* and lacks exons 1 and 2, confirming our hypothesis. Interestingly, the *LAGE2* gene has also been duplicated along with part of *NEMO*. However, a similar, but single-copy, *LAGE1* gene lies telomeric to the two *NEMO/LAGE2* duplicons. Sequencing the entire 120 kb from *NEMO* to *LAGE1* showed that the duplication spans 35.5 kb, with a single-copy 22-kb sequence separating the two copies. Analysis of great apes indicated that the *NEMO/LAGE2* duplication occurred before divergence of the human-chimp-gorilla lineages, around 10-15 million years ago. Despite this substantial evolutionary history, only 22 single nucleotide differences were found between the two duplicons, making them >99% identical. We have detected evidence of sequence exchange between the two duplicons, possibly pointing to gene conversion events. The homology and physical orientations of the two duplicons, and of smaller sections within each copy, lead to various alterations. We have detected four types of rearrangements that involved one or both of the *NEMO/LAGE2* copies or *LAGE1* as well. Together, these data describe an unusually complex genomic region that predisposes to various rearrangements, including one that causes the lethality associated with IP. These findings also emphasize that polymorphic duplications may be more common in the genome than currently perceived.

**Organization of LINE 1 sequences on human chromosome 21.** *H.D. Beris<sup>1</sup>, M.J. Roy<sup>1</sup>, K.A. Davis<sup>1</sup>, C. Lucero<sup>1</sup>, S.L. Carnahan<sup>1</sup>, A.E. Burket<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. Evidence suggests that L1 elements preferentially insert into A-T rich regions which are abundant in the heterochromatin of acrocentric p arms. Organization of L1s in heterochromatin and chromosome specific L1 structures are not well characterized. Southern blots of genomic and HC21 hybrid cell DNAs probed with the four L1 sub-fragments show HC21 specific patterns of L1 organization. Reverse Southern to L1 sub-fragments show that L1s on HC21 are more truncated than in the overall genome. Blots of HC21 hybrid cell mapping panel DNAs probed with the four L1 sub-fragments reveal L1s are highly under-represented on 21p. Most 21p copies are full length, polymorphic in number and organization, with substantially more full length L1s on 21p than 21q. We find that there may be as many as 40 full length L1s on 21p. HC21q represents 1.05% of the genome, and proportionately, should contain about 1% of all L1 elements. However, genomic sequence analysis shows that about 2% of all L1s are on 21q, suggesting that L1 is over-represented on this chromosome. Similarly, about 30 of the genome's 3000 full length L1s should be found on 21q. Sequence analysis shows only four full length elements on 21q. All four have target site duplications, and none belong to the recently active Ta subset of L1. L1s on 21q tend to be truncated at a limited number of different sites. Pulsed field mapping shows no full length L1s in the centromere region, but some 5' truncated L1s are contiguous to the D21Z1 alphoid cluster. Functional constraints may prevent L1 integration into the centromere. Since most full length 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. This concentration of full length L1s on 21p may facilitate p arm interactions among acrocentric chromosomes, increasing the risk of translocations and non-disjunction.

**The Human Genome: Never a dull chromosome, Never a dull band, Never a dull moment.** *X-N. Chen<sup>1</sup>, P. Bhattacharyya<sup>1</sup>, SY. Zhao<sup>2</sup>, M. Sekhon<sup>3</sup>, J. McPherson<sup>3</sup>, M. Wang<sup>4</sup>, U-J. Kim<sup>4</sup>, H. Shizuya<sup>4</sup>, M. Simon<sup>4</sup>, J.R. Korenberg<sup>1</sup>.* 1) Dept Medical Gen, Ste 2069, Cedars-Sinai Med Ctr, UCLA, Los Angeles, CA; 2) TIGR, Rockville, MD; 3) Washington Univ Genome Sequencing Ctr, St Louis, MI; 4) Caltech, Pasadena, CA.

The story of metazoan evolution is a story of genomic duplication. Primates are not an exception and the human genome reflects a rich history of recent duplication events that are a source of contemporary genomic variability and instability. We now link these duplicated regions to the draft sequence and show that they are located throughout chromosome arms, reflect regions of instability and represent gaps in the current draft sequence of the human genome. To avoid biases in sequence sets introduced by unstable regions, we have defined at random a subset of BACs for putatively duplicated regions and integrated them with the draft sequence. They provide anchor points for sequencing centromeres, pericentromeres and duplications in chromosome arms. These include a total of 6,000 BACs mapped by FISH, 3,500 defined at random, 184 from screens with alpha satellite, 346 with telomeric oligos and ~2,000 from other screens of the Caltech BAC libraries A and B. About 957 are STS linked. Out of 6,000 BACs, 373 mapped to centromeric regions, 192 to single centromeres, 150 to multiples and 20 to all human centromeres. Of 990 multisite BACs, 350 were defined at random suggesting a minimum of 10% of the genome was duplicated and interspersed. A total of 489 were fingerprinted, 33 with 5-29 bands showed no database match and suggested a minimum of 8% of duplications (non centromeric) were not represented in the fingerprint database. Of the 434 end sequenced BACs, 134 or 30% had no match in the draft sequence; 145 had hits of over 98% homology and 147 had hits of 80-98%. Three were located on orphan contigs. This suggested that at least 65% of the multisite clones detected repeated regions which were not included in the draft sequence. These BACs provide anchors for defining hotspots of genomic instability, for sequencing centromeric regions containing genes and for filling gaps in the draft sequence.

**Ancient Duplication: Chromosome 21 is paralogous to chromosome 11 in regions associated with DS phenotypes.**

*J.R. Korenberg, P. Bhattacharyya, G.M. Barlow, X.N. Chen.* Div Medical Gen, Cedars-Sinai Medical Ctr, Los Angeles, CA.

Genomic Duplication is the sustaining force of evolution. The emergence of the draft sequence of the human and other genomes provides the opportunity to trace these evolutionary events with respect to phylogenetic changes in metazoan evolution. We present evidence from in silico analyses to suggest that much of chromosome 21 is paralogous to chromosome 11 bands q23-q25 and results from genomic duplications that occurred during early vertebrate evolution before the emergence of Amphibians but after the emergence of bony fishes (Osteichthyes). At least four paralogous pairs were duplicated prior to and three after this period. Analyses of 127 cDNAs with accession numbers assigned to chromosome 21 yielded a total of 42 with homology to chromosome 11 (40/42 hits-1-3) and 20 with homology to cDNAs mapping to other genomic regions, 9 to second loci on 21, and no other region having more than 8 matches. Of the 127, 17 were detected by BLASTN, 24 by BLASTP and three to genomic draft sequence only. Eleven of the 17 detected by BLASTP shared domains. Clear homologs to both members were defined in all mammalian species examined. To evaluate the likelihood of duplication in Zebrafish, 27 chromosome 21/ chromosome 11 paralogous pairs were used to search the EST database. Of these, 18 of the HSA21 and 15 of the HSA11 members revealed homology with  $\geq 1$  EST. Of the 12 pairs with independent hits, 9 matched the same EST and two of the three with different hits were accounted for by clear preceding duplications. Although synteny is only locally maintained (2 Mb regions), paralogous regions are largely restricted to three bands on 11 and 21q11.2-22.3. These results illustrate a myriad of genomic mechanisms operating over about 400 million years of evolutionary change, provide evidence for high degree of expressed sequence preservation and an approach to determining the genes responsible for the successful rise of the tetrapods.

**Annotation of Human Chromosome 7 for Disease Research.** *S.W. Scherer, J. Cheung, K. Nakabayashi, J. Vincent, E. Petek, J.A. Herbrick, M. Li, J. Skaug, D. Kolozsvari, P. Deb-Rinker, A. Carson, A. Hudek, A. Paterson, L. Osborne, J. Rommens, A. Boright, L.C. Tsui.* Department of Genetics and The Centre for Applied Genomics, The Hospital for Sick Children, Department of Molecular and Medical Genetics, University of Toronto, Ontario, M5G 1X8, Canada.

The aim of our project is to establish a fully annotated genetic, physical, DNA sequence, and gene map of human chromosome 7. As part of this annotation we are also collecting and characterizing all clinical, functional, and biological information relevant to chromosome 7. Through analysis of DNA sequence data in the public and Celera databases we could detect corresponding genomic fragments for over 98% (6,500 of 6,579) of our collection of STSs known to map to chromosome 7. Initially, 65 large (>100 kb) DNA sequence contigs could be established covering >145 Mb of DNA. The average length was 1.68 Mb and the largest was 14 Mb on 7p. Our analysis indicates there are 10 known physical gaps not bridged by any clones and 55 sequence gaps spanned by known clones which are being sequenced (the statistics do not include the immediate 1-2 Mb flanking the centromere which require additional work). We are estimating the sizes of the physical gaps using FISH analysis and attempting to close them through screening additional cosmid, PAC, and BAC libraries and by performing long PCR. Moreover, a 100-200 kb duplicon at 7p11.1 was identified to be present in 30 copies elsewhere in the genome and another 200 kb segment was found in 3-4 copies at 7q11.23 and 7q22, further complicating assemblies. We have also identified genomic polymorphism on the long arm of chromosome 7. With the current DNA sequence map we have, so far, identified over 720 full-length genes and 1382 additional transcriptional units (total > 2000) on chromosome 7. Five genes larger than 1.0 Mb have been identified. Also, 150 rearrangement breakpoints, 700 FISH mapped clones, 3 fragile sites, and 3 imprinted regions could be accurately placed on the DNA sequence-based map. The integration of all biological information around the DNA sequence map will facilitate disease research.

**Alpha-Satellite Organization In Great Apes.** *M. Rocchi, C. Spalluto, S. Piccininni, L. Anelli, N. Archidiacono, R. Marzella, L. Viggiano.* DAPEG - Sezione Di Genetica, Univ Di Bari, Bari, Italy.

We have previously reported the comparative mapping of human alphoid sequences in great apes (Archidiacono et al., 1995). The unexpected results showed that the large majority of human probes do not recognize their corresponding centromeres, revealing a very rapid evolution of these sequences. Furthermore, these studies indicated that the suprafamily organization of alphoid sequences in great apes does not necessarily match that found in HSA. This suggests that alphoid centromeric sequences underwent a very rapid evolution. These findings prompted us to undertake a systematic cloning of alphoid probes in great apes to investigate their suprafamily organization and evolutionary history. Very few alphoid sequences from these species, have been characterized before. A further appealing reason to study the organization of alphoid sequences in great apes is the elucidation of their evolutionary relationship with pericentromeric regions, which we have shown to be extremely plastic (Jackson et al., 1999-2000; Eichler et al. 1999). We undertook the systematic cloning of alpha-satellite DNA sequences in the great apes for the following reasons: 1) to establish the suprafamily organization of alpha-satellite DNA in great apes; 2) to investigate the evolutionary history of this type of sequence in primates; 3) to establish the correlation between alpha-satellite DNA sequences and the distribution of NF-1 pseudogenes in primates. We report here the cloning of 36 alphoid probes derived from PTR centromeres using three different experimental approaches. These probes have been used in FISH experiments at high and low stringency hybridization conditions to investigate their suprafamily organization. We are able to group these alpha-satellite DNA sequences into two, not so clearly separated, large groups, as is also found in orang-utan (Haaf et al., 1998). In conclusion we think the suprafamily organization of alpha-satellite DNA in great apes is equidistant between the human suprafamily organization (every chromosome has at least one specific subset) and the suprafamily organization of Old World Monkey (OWM) (only one alpha-satellite DNA sequence).

**Genomics and Evolution of the Human X Chromosome Centromere.** *M.G. Schueler, H.F. Willard.* Case Western Reserve University, Cleveland, Ohio.

Pericentromeric regions of human chromosomes are largely uncharacterized but contain sequences involved in centromere function. Genomic analysis of these regions may reveal evolutionary events that have led to current mammalian chromosome structure and detect DNA elements common to functional centromeres. We report a BAC/PAC contig joining the X chromosome-specific alpha satellite array, DXZ1, with the most proximal expressed gene in Xp11.21, ZXDA. Our contig spans ~ 500 kb represented in 19 clones. The region consists of monomeric alpha satellite, gamma satellite, interspersed repeats and a novel 35 bp repeat, but lacks large segments of unique or paralogous sequences. The junction between arm-specific sequences and centromeric satellites is located 149 kb from the ZXDA gene and is marked by an L1M1 LINE element adjoining a partial alpha satellite monomer. This unique map resource allows us to test and formulate hypotheses regarding pericentromeric structure and evolution. The most distal alpha satellite in this region lacks higher-order structure and CENP-B boxes; however, we have identified diverged higher-order sequences at the border of the DXZ1 array that provide direct evidence of unequal crossover as a mechanism of evolution of alpha satellite arrays. Second, DXZ1 and diverged DXZ1 sequences within this ~ 20 kb transitional region at the array border are phylogenetically distinct from the monomeric alpha satellite that adjoins the chromosome arm. Third, L1 dating predicts that monomeric alpha satellite in the region has resided at this location since early in primate evolution. Further, in a screen of ~ 4.5 Mb of DXZ1 DNA, only the currently active L1Hs element was identified, indicating a more recent origin for the DXZ1 array. Functional studies indicate that DXZ1 alpha satellite can form active, de novo centromeres (see abstract by Rudd). It is our hypothesis, therefore, that monomeric alpha satellite is a remnant of the ancestral primate X centromere and that the sequences currently functioning as the X centromere have evolved recently. Conservation of X chromosome content through evolution makes our contig an excellent model upon which to conduct comparative analysis of centromere evolution.

**Alpha satellite organization in chimpanzee (*Pan troglodytes*, PTR).** *L. Viggiano, C. Spalluto, S. Piccininni, L. Anelli, N. Archidiacono, R. Marzella, M. Rocchi.* Genetics, Univ of Bari, Bari, Italy.

We have recently reported the comparative mapping of human alphoid sequences in Great Apes (Archidiacono et al., *Genomics* 25:477-484 1995). The unexpected results showed that the large majority of human probes do not recognize their corresponding centromeres, revealing a very rapid evolution of these sequences. Furthermore, these studies indicated that the suprafamily organization of alphoid sequences in Great Apes does not necessarily match that found in HSA. These findings prompted us to undertake a systematic cloning and characterization of alphoid probes in Great Apes to investigate their suprafamily organization and evolutionary history. Very few alphoid sequences from these species have been indeed cloned and characterized so far. A further reason to study the organization of alphoid sequences in great apes is the elucidation of their evolutionary relationship with pericentromeric regions, which we have shown to be extremely plastic (Jackson et al. *Hum.Mol.Genet.* 8:205-215, 1999; Eichler et al., *Genome Res.* 9:1048-1058, 1999). We report here the cloning of 36 independent alphoid probes derived from PTR centromeres using three different experimental approaches. These probes have been used in FISH experiments at high and low stringency hybridization conditions to investigate their suprafamily organization. Moreover the most representative clones have been sequenced and compared against the human alpha-satellite DNA consensus sequence (Romanova et al., 1996). Our analysis have shown that a distinct and strict suprafamily organization, as found in humans, does not exist in PTR. We hypothesize that the chromosomal organization of alphoid sequences in this species is intermediate between the highly chromosome-specificity and strict suprafamily distinctions as occurs in humans, and the simple organization of Old World Monkey (OWM) where one alpha-satellite DNA sequence is supposed to be shared by all chromosomes.



**The map problem: a comparison of genetic and sequence-based physical maps.** *A.T. DeWan<sup>1</sup>, A.R. Parrado<sup>1</sup>, T.C. Matisse<sup>2</sup>, S.M. Leal<sup>1</sup>.* 1) Laboratory of Statistical Genetics, The Rockefeller University, New York, NY; 2) Department of Genetics, Rutgers University, Piscataway, NJ.

The genetic order of autosomal markers from Marshfield panel 10 was compared to their physical order based upon the assembled non-redundant human genome sequence from the Human Genome Project - Santa Cruz (HGP-sc) and Celera (CEL) databases. The likelihoods were calculated for the minimum recombination maps for each of the 22 autosomes and compared to the likelihoods for alternative map orders, to determine which markers could be placed on the genetic maps based upon a likelihood ratio criterion of 1000:1. Of the 380 autosomal panel 10 markers which are spread across the genome at ~10cM intervals, a total of 254 (67%) and 265 (70%) markers could be found in the HGP-sc and CEL databases, respectively. Through the use of e-pcr, an additional 42 (11%) of the markers could be placed on the assembled genome sequence from the HGP-sc database for a total of 296 (78%) markers. E-pcr could not be performed on CEL sequence data, due to unavailability. A total of 210 (55%) of the markers could be found in both databases. There was an inconsistency in the order of 9 (3%) and 15 (6%) of the markers when the genetic order was compared to their order in HGP-sc and CEL, respectively. Only two of these inconsistent markers were redundant in HGP-sc and CEL. For all of the inconsistent markers only 1/9 of the HGP-sc markers and 1/15 of the CEL markers was the genetic order not supported by a likelihood ratio of 1000:1. In addition, for 2/9 HGP-sc and 2/15 CEL markers the physical chromosomal assignment did not match the genetic chromosomal assignment. Of the 15 inconsistent CEL markers, 10 were located in the HGP-sc database. The HGP-sc physical order and genetic order were consistent for 8 of these 10 markers. Likewise 6/9 of the inconsistent HGP-sc markers were identified in the CEL database. The CEL physical order and genetic order were consistent for 4 of these 6 markers. In conclusion, the majority of the inconsistencies between the physical and genetic map order point to errors in the physical map order.

**Haplotype analyses of the HLA-DRB1 region suggest a high mutation rate in the DRB1 gene.** *H. Hohjoh, J. Ohashi, K. Tokunaga.* Human Gen/Grad School Medicine, Univ Tokyo, Tokyo, Japan.

Phylogenetic trees for the HLA-DRB1 alleles suggest that DRB1\*0701 may have diverged from other DRB1 alleles before the separation of human and chimpanzee. However, based on nucleotide changes in the PRKRA pseudogene specifically present in the HLA-DR53 group, the divergence times of the DRB1\*0701 and DRB1\*04 alleles linked with PRKRA pseudogene were estimated to be 0.38~0.3 million years (Myr) ago. To settle this issue, here we study haplotypes with the centromeric flanking region of DRB1, DRB1, and PRKRA pseudogene. Results indicate that the haplotypes carrying DRB1\*0701 and DRB1\*04 have been derived from a common ancestral haplotype, and that the divergence of the haplotypes may have occurred 0.92~0.37 Myr ago. In addition to the above results, the haplotypes further reveal that the number of selectively neutral (synonymous) substitutions in DRB1 is significantly higher than those in the centromeric flanking region and PRKRA pseudogene, although the balancing selection may have acted equally throughout the haplotypes. This suggests that DRB1 has a significantly high mutation rate. Such a high mutation rate as well as the balancing selection may contribute to the highly complex polymorphism in DRB1.

**A panel of somatic cell hybrids specific for the Olive baboon (*Papio anubis*, Old World Monkeys).** *R. Marzella, V. Miolla, P. Chiarappa, C. Carrozzo, M. Rocchi, N. Archidiacono.* DAPEG Sezione di Genetica, Univ Bari, Bari, Italy.

A draft of the human genome sequence has been accomplished moving the Human Genome Project into its postgenomic era. It is becoming evident, however, that the understanding of our humanness will be achieved only by comparing our genome and its functional aspects with those of our closest relatives: the primates. Unfortunately the dearth of primate resources for molecular analyses is very serious. In recent years we have investigated the plasticity of the pericentromeric regions of human chromosomes (Jackson et al. *Hum.Mol.Genet.* 8:205-215, 1999; Eichler et al., *Genome Res.* 9:1048-1058, 1999). This task have been primarily achieved by comparative mapping of appropriate human probes on cytological preparation of primates. Molecular aspects of these studies, however, are severely handicapped by the lack of appropriate molecular tools, chromosome-specific resources in particular. For this reason we have initiated the construction of a panel of somatic cell hybrids specific for a selected primate species. Here we report the preliminary characterization of a panel of somatic cell hybrids specific for the Olive baboon (*Papio anubis*, Old World Monkeys). This is one of the few primate species for which a public available BAC library exists (P. de Jong, <http://www.chori.org/bacpac/>). The B14-150 CHO cell line (TK-) was used as a rodent parental cell line. The characterization of the baboon chromosomes retained in each hybrid was first obtained by banding analysis. The DNA from each hybrid was selectively amplified by dual-Alu primers and the amplified products were then used as a probe in FISH experiments on both baboon and human metaphases. The reverse-painting characterization also allowed the identification of chromosomal fragments, that are a non rare occurrence in somatic cell hybrids. The work is in progress. At present the following chromosomes have been found to be retained in various combinations: Whole chromosomes 6, 10, 12, 20, 17, 19 , 13 , and Y; fragments of chromosomes: 1, 2, 5, 7, 8, 10, 13, 17.

**Construction and application of BAC libraries constructed using sheared DNA.** *K. Osoegawa, C.-L. Shu, P.J. de Jong.* Children's Hospital Oakland Research Institute, Oakland, CA.

Bacterial artificial chromosome (BAC) libraries have initially been developed for use in genome mapping and sequencing. After completion of the human draft sequence, mapped and sequenced BACs have become important tools for disease diagnostics and functional genomics. Nevertheless, there is a need for additional human clones to facilitate genomic gap closure. We developed procedures for cloning sheared DNA in a modified BAC vector with the expectation to avoid cloning bias due to aberrant levels of the restriction sites in specific genomic regions. BACs created from sheared DNA will also be useful to determine more random end sequences to assist in scaffolding sequence contigs derived from whole genome shotgun sequencing. BAC libraries with different average insert sizes and random ends support the now accepted hybrid approach based on a combination of whole genome shotgun and clone-by-clone sequencing. To create the required BACs, HMW DNA is sheared by multiple cycles of freezing and thawing. The fragment ends are blunted by subsequent treatments with Mung Bean nuclease and T4 DNA polymerase, and are then ligated to the blunt-end side of an adapter that also has a 3' overhang (ACAC). The ligation products are size-fractionated to remove the excess adapter and to obtain the clones in the desirable size range. The new vector (pTARBAC6) has two BstXI restriction sites flanking a replaceable stuffer fragment. Upon BstXI digestion, a vector fragment with two 3' overhangs (GTGT) is generated, complementary to the genomic DNA. We have been able to construct several BAC libraries from *Drosophila*, *Ciona savignyi* and mouse with different average insert sizes to fit the applications. Provisional results with the new libraries indicate random clone distribution and a very low level of undesirable chimeric clones. Initial screening results for the fly BAC library indicate a possible extension of contigs towards the telomeres. Information on our libraries can be obtained at: [www.chori.org/bacpac](http://www.chori.org/bacpac). Library construction was supported either directly or indirectly by grants from the NHGRI. The US DOE specifically funded the technology development of sheared BAC libraries.

Program Nr: 1607 from the 2001 ASHG Annual Meeting

**A new resource of chimpanzee biomaterials: gender determination and genotyping data.** *R. Robledo, C.M. Beiswanger, P.K. Bender, W. Beggs, J.C. Leonard, R.Z. Goldman, M.J. Manduka, J. Coccia, J.C. Beck.* Coriell Inst Medical Research, Camden, NJ.

Recognizing the need for resources to permit genetic comparisons of closely related species, the Coriell Cell Repositories, in collaboration with the Yerkes Regional Primate Research Center, have begun the establishment of a collection of biomaterials from chimpanzees (*Pan troglodytes*). Lymphoblastoid cell lines were established from blood collected in ACD tubes using standard protocols with Epstein-Barr virus and phytohemagglutinin (PHA). Fibroblast cell lines were established from skin biopsies. All established lines are viable and contaminant-free. Karyotype analyses confirm that the lines are chimpanzee. In addition, the chimpanzees were genotyped using primers from the ABI PRISM Linkage Mapping Set, version 2 and probes discriminating gender based on X chromosome amelogenin alleles. Even though the markers tested were developed for analysis of human loci, all markers successfully amplified chimpanzee DNA. Some markers show no overlap in allele size between chimpanzees and humans, while others show a substantial overlap with humans tending to have larger allele sizes than chimpanzees. Only one marker (D22S539) proved to be mono-morphic in these chimpanzees, a finding not surprising in view of the low heterozygosity reported in humans. The amelogenin probes correctly identified the gender of all samples and the microsatellite data are consistent with the proposed pedigrees derived from behavioral studies. These data show that it is possible to use the ABI PRISM Linkage Mapping Set for a wide genome screening for genetic mapping, evolutionary studies and paternity testing. Detailed information about these samples, including ordering instructions, is available in an electronic catalog (<http://locus.umdj.edu/ccr>). Supported in part by NIH/NCRR, RR-000165.

**BAC Libraries From Non-human Primates and Other Model Organisms for Comparative Genome Analysis.** *B. Zhu, K. Osoegawa, C. Shu, P. deJong.* BACPAC Resources, CHORI, Children's Hospital Oakland, Oakland, CA.

With the increasing interest in comparative genomics, BAC libraries have become important resources permitting the isolation of regions of syntenicity between species through BAC contigs. Intraspecies comparisons do not rely on BACs but can also be derived by long PCR. Interspecies comparisons cannot be done by PCR - except for very closely related species. Genomic comparison for syntenic regions thus relies on selective cloning approaches or by selecting clones from total-genome libraries. We have recently developed hybrid BAC/YAC vectors ("pTARBAC") to create the newer BAC libraries. BAC clones created in the hybrid vectors through cloning in *E. coli*, can be deleted individually with restriction enzymes. The deleted clones can be repaired to original size by homologous recombination in yeast between uncloned high molecular weight DNA from the same species and the linear deleted BAC. This process occurs following co-transformation of the linear vector and the deleted BAC DNA and has therefore been labeled as "Transformation Associated-Recombination" by Larionov & Kouprina. To permit this application, we constructed new BAC libraries in pTARBAC vectors for rhesus macaque, chimpanzee, baboon, rat and zebrafish. Preliminary characterization showed that the Rhesus Macaque (CHORI-250) and Chimpanzee (CHORI-251) BAC libraries have at least 10-fold genome redundancy with average inserts of about 175 Kb. The Baboon BAC (RPCI-41 male) library has already been arrayed into 384-well plates and represents 10-fold genome coverage, with insert size of 174 Kb. The zebrafish BAC library (CHORI-211) has been arrayed into 384-well plates at about 10-fold genome coverage with an average insert size of 165 Kb. A 10-fold redundant new rat (BN/SsNHsd/MCW) BAC library has just been completed. Characterization of the first 5-fold redundancy part of the rat library indicates average inserts of 214 kb. Information about BAC libraries, vectors and procedures is available from our website: [www.chori.org/bacpac](http://www.chori.org/bacpac). The new BAC libraries constructed have been supported by grants from NHGRI, the US DOE and through sub-contracts.

**An integrated physical map of the human and chimpanzee genomes.** *M. Sekhon.* Genetics/GSC, Washington University, Saint Louis, MO.

The genomes of Chimpanzee (*Pan troglodytes*) and Humans (*Homo sapiens*) are believed to be 98% identical at the nucleotide level. In order to further investigate the similarities and differences between the two organisms, we attempted 68,721 fingerprints of a Male Chimpanzee BAC library ([www.chori.org/bacpac/](http://www.chori.org/bacpac/)) and integrated the fingerprint data into our BAC fingerprint physical map of the Human genome ([http://genome.wustl.edu/gsc/human/human\\_database.shtml](http://genome.wustl.edu/gsc/human/human_database.shtml)). To date, we have been able to position ~67% of the chimpanzee clones to corresponding mapped BACs of the human genome with identical or highly similar restriction digest fingerprint patterns. The correct placement of the chimpanzee BAC clones in the human physical map is being assessed by sequence analysis and fluorescence in situ hybridization (FISH) of a subset of the mapped clones. The Chimpanzee mapping data is a valuable resource for selecting clones of interest for sequence comparison with Human draft and finished data. Human disease regions in the physical map can be identified and corresponding chimpanzee clones can be selected for sequencing and further analysis. In addition, chimpanzee clones that cannot be placed on the human physical map are being further investigated to see if they represent regions not present in the human genome.

**Using DAML format for representation and integration of complex gene networks: implications in novel drug discovery.** *K. Baclawski*<sup>1,2</sup>, *E. Neumann*<sup>2,3</sup>, *T. Niu*<sup>1,4</sup>. 1) College of Computer Science, Northeastern University, Boston, MA; 2) Jarg Corporation, Waltham, MA; 3) Beyond Genomics, Inc., Waltham, MA; 4) Program for Population Genetics, Harvard School of Public Health, Boston, MA.

Great strides have been made in understanding the complex molecular networks underlying biological systems. Advances in high-throughput microchip-based assays are providing us with global gene activity profiles characterizing the output of the gene regulatory network. The eXtensible Markup Language (XML) is becoming a key component in data exchange in biology and currently there are over a dozen XML DTDs and schemas for bioinformatic applications, including BioML, CellML, RNAML, DDBJ-ML and BSML. However, there are intrinsic limitations of using XML in representing biopathways because of its hierarchical nature. To address this problem, we introduced a new way of knowledge representation using DARPA Agent Markup Language (DAML) schemas through ontology-based models for integration of complex genetic networks. This new approach has overcome the restriction of the use of hierarchical data structure, and can facilitate automatic retrieval and update of gene network-related data and can be transformed into other XML dialects. Our prototype is expected to shed novel insights on genetic networks that will help our efforts towards better understanding of complex disease etiology, which in turn, can have significant expectations in clinical pharmacogenomics.



**Reproducibility and Selection of Probe Sets Across Generations of Microarrays.** *A. Nimgaonkar<sup>1</sup>, D. Sanoudou<sup>2</sup>, A. Butte<sup>1</sup>, J.N. Haslett<sup>2</sup>, L.M. Kunkel<sup>2</sup>, A.H. Beggs<sup>2</sup>, I.S. Kohane<sup>1</sup>.* 1) Informatics Program, Children's hospital, Harvard Medical School, Boston, MA; 2) Genetics Division, Children's Hospital, Harvard Medical School, Boston, MA.

The availability of massively parallel expression profiling is changing the scientific paradigms of biological investigation, yet the rapidity of change of these technologies present large challenges. Commercial microarrays are regularly modified with new sets of genes and improved target sequences. Comparing datasets across generations is crucial for any long-term research project. However, to date, there are no means to allow comparisons across generations. We measured the reproducibility of gene expression level across two generations of microarrays (HuGene FL and HG-U95A, 7129 and 12,626 probe sets respectively) produced by Affymetrix. Four normal muscle samples were hybridized on one HuGene FL and one HG-U95A chip each. The 7,129 HuGeneFL probe sets were compared to the 8,075 probe sets on the HG-U95A chips that represent the same gene. The four transcriptome correlations ( $r^2$ ) across the two microarray generations varied from 0.58 to 0.70. Probe sets with 3 or more probe pairs in common had a correlation greater than 0.75, and considering those with 13 or more probe pairs in common improved the correlation to over 0.90. Probe sets with no probe pairs in common decreased the correlation to 0.27. When particular types of probe sets were considered (e.g. probe sets for which Affymetrix was not possible to pick a full set of unique probes), the correlation changed statistically significantly. Considering each gene's four measurements across the two generations, around 20% of probe sets were negatively correlated. That is, the expression level of these genes changed in opposite directions when measured across the two chip generations.

Our results support limited comparability between microarray generations, and introduce computational methods that will enable such comparisons. Further study of more samples and tissue types could establish a widely applicable analytical model to make the most of current datasets, and accelerate work with future microarray generations and platforms.

**Using Genetic, Radiation Hybrid, and Sequence Maps Comparisons to Analyze Recombination and Gene Density.** *J.E. Stajich, E.R. Hauser, J.M. Vance.* Center for Human Genetics, Duke Univ Medical Center, Durham, NC.

The completion of a rough draft assembly of the human genome provides many opportunities to revisit established observations of genomic trends. We have compared the recently completed sequence maps with established genetic and RH maps to investigate relationships of map distance and sequence properties. Our research studied the human genome on a 100kb scale, and compared gene density and repetitive elements with recombination rates.

The sequence based marker comparisons were constructed using e-PCR on the Golden Path of the human genome as constructed by UCSC. Repeat density was calculated by RepeatMasker and tandemrepeatfinder on each segment of genomic sequence. Confirmed genes from Project Ensembl were used to score the number of genes in a given segment to assess number of genes in a location.

Comparisons of genetic and sequence distances across a chromosome can be used to infer variation in recombination rates. A model relating genetic distance to sequence distance was developed to find correlations in recombination and sequence statistics such as gene and repetitive element density. We found strong positive correlation of SINE elements to gene density and moderately negative correlation of LINE content to gene density in a region.

The application of the map integration aspect of this project is useful in aiding candidate gene discovery from linkage analysis results. Software was developed to automate the integration of genetic and radiation hybrid markers onto a single map to provide researchers with a simple interface for locating candidate sequence regions. Ultimately with this approach genes can be easily mined automatically from databases such as Ensembl using only markers from linkage analysis studies to identify the regions.

**Methods for Completion of a Human Physical Map.** *S. Jaeger.* Genetics/GCS, Washington University, Saint Louis, MO.

As part of the Human Genome Project, we have constructed a physical map of the human genome comprised of greater than 400,000 bacterial artificial chromosome (BAC) clones, primarily from the RPCI-11 library (BAC/PAC Resources). Each clone was digested using HindIII and the fragments were separated by gel electrophoresis. Bands were identified using IMAGE software (Sanger Centre), and the resulting fingerprint was put into a Fingerprint Contig (FPC) database (Sanger Centre). Clones binned and initially ordered using the FPC software tools were manually edited to form contigs. Contigs were localized to the 24 chromosomes using various marker data, hybridization assays, FISH, and *in silico* PCR. Currently, the BAC map contains ~750 contigs. Many clones for the working draft of the human genome were selected using this map and the entire working draft sequence has been aligned to it. Remaining gaps in the map are likely due to biases in the BAC libraries and repetitive regions. We are using several techniques to close the gaps, and complete the map. One method for sizing a gap is FISH. Clones from both ends of the gap are fluorescently labeled and hybridized to chromosome fibers. The gap can be sized by measuring related signal lengths. If the gap is <~30kb, primers can be selected using end, draft, or finished sequences (TIGR database, NCBI) from clones flanking the gap, and a PCR-based method can be used to bridge the gap. A method for closing a larger gap is endwalking. BAC end, draft or finished sequence is used to develop probes. Radioactively labeled probes are hybridized to filters containing clones from RPCI-13 and RPCI-11 Segment 5. Clones identified from the hybridization are fingerprinted, incorporated into FPC, and used to extend the ends of the contig. Some clones extend the contig without filling the gap. In this case, those clones are chosen for end sequencing by the Physical Mapping Group. Those sequences are used to develop probes and do further walking. Yeast artificial chromosome (YAC) clones are chosen that span gaps that are not readily closed by walking. The spanning YACs are used to generate further probes and may also be used directly for sequencing templates.

**The identification of disease genes by genomic sequencing analysis of 8q22-q24.** *N. Shimizu, A. Shimizu, T. Sasaki, A. Shintani, K. Kawasaki, J. Kudoh, S. Minoshima, S. Asakawa.* Dept Molecular Biol, Keio Univ Sch Medicine, Tokyo, Japan.

Recently, the initial draft sequence was reported by the international human genome sequencing consortium including us, but further efforts will be needed to extract the full information contained in the human genome. For this task, we have focused on the human chromosome 8q22-q24 in which at least five disease genes responsible for developing glaucoma, Cohen syndrome, Klippel-Feil syndrome, Langer-Giedion syndrome, and myoclonic epilepsy have been mapped. The 8q22-q24 (D8S1822-D8S1826) region is about 30 Mb, which we covered with 7 contigs of Keio BAC clones. These BAC clones have been processed for shotgun sequencing in combination with primer walking. We have finished sequencing of 82 BAC clones, covering about 40% of the 8q22-q24 region (~ 10 Mb) with the longest continuous sequence of 2.1 Mb. Genomic sequence was subjected to homology search with the database. We have so far identified 112 genes, including 73 known genes (PGCP, LC27, MATN2, RPL30, UK114, POP1, PRO1097, KRS1, GEM, CDH17, PABP, HTPHLP, P53R2, HYD, ODFPG, TIEG, DPYS, ST7, OXR1, INT6, ANGPT1, DC6, EBAG9, TRPS1, EXT1, EIF3S37 etc.) and 15 novel genes (C8orfK1, C8orfK2, C8orfK3, C8orfL4 etc.) in addition to 24 pseudogenes (SMT3H2P, LETM1P, GAPDP, MLF2P, RPL30P, RPL39P, SMT3H2P, EEF1A etc.). Initial characterization of these genes including mutation search for the patients of epilepsy and Cohen syndrome will be presented.

**Genome Database, GDB, post-sequence advances.** *A.J. Cuticchia<sup>1</sup>, C.J. Porter<sup>1</sup>, W. Zhu<sup>1</sup>, C.C. Talbot Jr.<sup>2</sup>.* 1) Bioinformatics Supercomputing Centre, The Hospital for Sick Children, Toronto, ON, Canada; 2) Genome Database, The Johns Hopkins University School of Medicine, Baltimore MD, USA.

Since its inception in 1990, the Genome Database (GDB) has provided free access to curated mapping and marker information generated by scientists worldwide. With the accelerated timetable of the Genome Project and subsequent publication of the draft sequence in 2001, GDB has, in parallel, increasingly developed sequence-based tools and techniques to incorporate sequence information with its traditional, e.g. linkage, RH, and FISH, mapping data. GDB now uses e-PCR to create maps comprising amplimers and SNPs that have been computationally positioned on both finished and draft sequence contigs, and updates these maps as their source contigs change. We can thereby integrate positional data from sequence more directly with other mapping data to update and refine our Comprehensive Maps.

A GDB-BLAST tool returns any GDB objects (clones, amplimers, and genes) associated with the returned sequence hits, and our e-PCR tool analyzes query sequences against GDB's many amplimers. The use of Toronto's Bioinformatic Supercomputing Centre's supercomputers has enabled GDB to develop a novel e-PCR Database. Our e-PCR Database Lookup tool essentially reverses the conventional e-PCR process and allows users to enter query amplimers and search GenBank for sequences that should, based on computation, serve as their templates.

We have recently devised a new method to link data from heterogeneous databases and incorporate them within the display of the associated GDB objects. Another new database has been created to store sequence position data for presentation either via this new technique or independently in its own browser. This new display method is used to access sequence context and position information, and to incorporate data from external databases of genome annotation with a GDB record. These links will also be used to present additional functional, expression, and proteomic data as they become available. Links have been put in place between amplimers and the GDB e-PCR Database to allow users to find sequences overlapping the markers' positions.

***MutationView* : A Graphical Database System for Mutations and Polymorphisms in Human Disease Genes.** S.

Minoshima<sup>1</sup>, M. Ohtsubo<sup>1</sup>, S. Mitsuyama<sup>1</sup>, S. Ohno<sup>1</sup>, T. Kawamura<sup>1</sup>, S. Ito<sup>2</sup>, F. Ito<sup>3</sup>, N. Shimizu<sup>1</sup>. 1) Dept Molec Biol, Keio Univ Sch Medicine, Tokyo, Japan; 2) Dept Environ Health Science, Setsunan Univ, Osaka, Japan; 3) Dept of Biochem, Setsunan Univ, Osaka, Japan.

More than 1000 disease-causing genes have been reported. Databases for mutations in these disease-causing genes are indispensable for the diagnostics, therapeutics and basic research of the diseases. At present, mutation data are maintained as individual Locus-specific Databases (LSDBs) for some 200 diseases and therefore more comprehensive database system with common graphical user-interface is required. We have established the *MutationView* as an integrated graphical mutation databases. Currently, the *MutationView* has collected 5563 entries of mutations/polymorphisms from 1091 literatures dealing with 183 genes involved in 173 distinct diseases. The characteristic features of the *MutationView* are as follows:

(1) Several ways to access are available through the chromosomal map of the gene or disease, anatomical chart of disease-associated organ or tissue, and diagram of causative gene product.

(2) Various data display and analysis functions are available such as 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.

(3) Mutation data can be put in any web server and therefore *MutationView* system is ideal to link global mutation databases managed by LSDB curators.

The user ID and password are issued upon formal applications through the URL <http://mutview.dmb.med.keio.ac.jp>. Computer demonstration will be performed at the meeting.

**Comprehensive setting of 30,000 polymorphic microsatellite markers throughout human genome.** *K. Okamoto<sup>1,2</sup>, S. Makino<sup>1</sup>, T. Endo<sup>3</sup>, H. Hayashi<sup>1</sup>, A. Oka<sup>1</sup>, K. Fujimoto<sup>1</sup>, A. Denda<sup>1</sup>, H. Watanabe<sup>1,2</sup>, E. Tokubo<sup>1</sup>, R. Sato<sup>1,2</sup>, A. Takaki<sup>1</sup>, Y. Sakurai<sup>1</sup>, Y. Nagatsuka<sup>1</sup>, T. Imanishi<sup>3</sup>, T. Gojobori<sup>3</sup>, K. Tokunaga<sup>4</sup>, G. Tamiya<sup>1</sup>, H. Inoko<sup>1</sup>.* 1) Sch Medicine, Tokai Univ, Isehara, Japan; 2) Chugai Pharmaceutical Co., Ltd, Gotemba, Japan; 3) National Institute of Genetics, Mishima, Japan; 4) Grad Sch Medicine, Univ Tokyo, Tokyo, Japan.

The aim of this study was to identify novel polymorphic microsatellite markers throughout human genome.

Our previous data indicated that microsatellite markers showed a linkage disequilibrium with disease-related alleles spanning from 100 to 200 kilobases (kb). Based on this evidence, we made a plan to identify a total of 30,000 polymorphic markers throughout human genome with 100 kb intervals. In addition to approximately 10,000 markers that were previously registered in GenBank, we identified 20,000 novel markers. First, we identified all available microsatellite sequences from the human genome draft sequence. In order to investigate the microsatellite polymorphism, we performed genotyping using pooled DNA from 100 healthy Japanese individuals. Approximately 30,000 markers were identified as a polymorphic in Japanese pool. Furthermore, we investigated the characteristics of these polymorphic markers, including heterozygosity, allele number, and length of repeated unit. Mean heterozygosity and allele number were approximately 0.65 and 6.3 respectively. These data strongly suggest that we did obtain highly informative genetic markers. Next, we have mapped newly identified polymorphic markers into chromosomes, composed of the assembled genomic sequences from the repository of International Human Genome Sequencing Consortium. With these mapping data, we plan to submit the our novel the polymorphic markers to the public genomic repository.

A total of 30,000 markers will aid the construction of a useful marker map of the human genome and will be a powerful tool for fine mapping of disease-susceptibility genes in genome-wide association studies. In addition, they should be applied for other purposes such as population studies.

**Identification of 140,000 cSNPs in the human genome.** *T. Tanaka*<sup>1,2</sup>, *Y. Ohnishi*<sup>1,2</sup>, *R. Yamada*<sup>1,2</sup>, *Y. Nakamura*<sup>1,2</sup>. 1) Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; 2) SNP Research Center, RIKEN, Tokyo, Japan.

SNP is the simplest and the most common form of DNA polymorphism. Owing to these characteristics, it is expected to be of great use in association studies on common diseases or drug sensitivities. In particular, those within promoter regions, exons and introns, which we call cSNPs, are much more valuable than others, since cSNPs have much higher probability to directly modify functions of genes or their spatial and temporal patterns of expression. On April 2000, we have launched a national project in Japan to identify 150,000 SNPs in two years. Our method is as follows; human genomic sequences are extracted from GenBank database and PCR primers are synthesized to amplify exons, promoter regions and introns. Genomic DNA from three independent individuals are mixed into one and used as templates for direct sequencing of PCR products. In total, 24 individuals are examined in eight sample tubes. Computer program Polyphred is used to assist verification of SNPs by eye inspection, followed by construction of SNP database open to public via world wide web (<http://snp.ims.u-tokyo.ac.jp>). We have already discovered 140,000 cSNPs in one and a half year. Approximately 20% of them are within exons. At present, only 25% of our SNPs were identical to those in dbSNP database. In addition, we have already identified 3676 cSNPs on chromosome 22, whose result is different from that of the previous report of 2730 SNPs on that chromosome. These may reflect the strategic differences to identify SNPs, problem on the reliability of the public database, or ethnic differences. With our catalog of 150,000 cSNPs in the human genome when completed, we believe further investigation should be much easier to identify genes related to common diseases or drug sensitivity.



**New labeling method for microarray hybridization detection.** *C. Xiang, M. Chen, M. Brownstein.* Lab of Genetics, NIMH/NIH, Bethesda, MD.

The methods that have been developed for routine labeling of cDNA probes for microarray studies require a minimum of 20 ug of total RNA or 2 ug of poly(A)RNA. Many of the tissue samples that one would like to study are small and hard to obtain. Consequently it has been difficult, if not impossible, to use them for expression profiling. Recently, methods for amplifying RNA and then labeling probes have been published. These multistep procedures are tedious and expensive to perform, however. Here we describe a new technique for preparing fluorescent probes from total RNA. It is based on random hexamer priming with oligonucleotides having bases with free amino groups along with incorporation of amine-modified bases into the cDNA produced by reverse transcription. Fluorescent dyes are added to the cDNA products chemically after they are formed. This addition is quite efficient, and the dyes are much cheaper to purchase than dye-labeled bases. The proprietary method described herein can be used to label as little as 0.5 ug of total RNA. When a single-stage RNA amplification procedure is employed in tandem with our method, it can be used to label RNA from 1000 or fewer cells.

**Potential for genetic factors in General Clinical Research Center (GCRC) studies.** *J.A. Phillips III<sup>1</sup>, D. Robertson<sup>2</sup>.*  
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Genetics is relevant to virtually all human diseases. While the genome sequence can be used to identify genes that may contribute to a disease, it cannot predict all possible genotype- phenotype correlations. Thus phenotype mapping will be needed to identify the genes that contribute to diseases. Many diseases are rare but some such as hypertension are common. Currently, little is known about genes that cause, or could be used to diagnose and treat such common diseases. We hypothesize that most diseases, phenotypes or clinical trials being studied in General Clinical Research Center (GCRC) research projects can be related to one or more known genes. To determine the proportion of GCRC projects for which a potential genetic factor could be identified, we reviewed 32 consecutive projects submitted to the Vanderbilt GCRC from 2/09-5/01/01. The phenotypes and/or medication(s) being studied were examined to identify candidate genes (major, modifying or multifactorial) or those which metabolize the medication being studied. We used the following databases: 1) NCBI (OMIM, LocusLink and SNP) at <http://www.ncbi.nlm.nih.gov/Sitemap/index.html> and 2) Human P450 Metabolism at [http://www.gentest.com/human\\_p450\\_database/srchh450.asp](http://www.gentest.com/human_p450_database/srchh450.asp). Of these 32 projects 59, 22, 6, 6 and 3% were medication trial, phenotyping, vaccine trial, gene identification and device testing. We identified genes which could potentially affect the phenotype being studied in 30/32 (94%) of the projects. The genes identified were of major effect, modifier/multi factorial or metabolized medications in 14, 43 or 43%, respectively of these 30 projects. Our data indicate that: 1) 94% of GCRC projects studied related to genes and 2) the most common study type (59%) is a medication trial for which gene(s) that metabolize the medication or modify the phenotype and have known variation were identified. Our data suggest that: 1) most GCRC projects are studies of phenotypes for which genes that could cause variation are known and 2) GCRCs have great potential to map phenotypes and become clinical arms of the Human Genome Project.

**A semi-automated data-processing system for large-scale determination of SNP allele frequency by SSCP. T.**

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Millions of candidate SNPs accumulated in public database are valuable resource for medical and biological studies, and technique to characterize these SNPs is required. We have developed a cost-effective and streamlined technique, PLACE-SSCP, in which the PCR products are fluorescently labeled and applied to capillary electrophoresis for SSCP-separation of SNP-alleles. SNPs are identified after sequencing of individuals who have different SNP-alleles and are correlated with the corresponding peaks of SNP-alleles. Allele frequencies of SNPs in the pooled DNA can be precisely determined from the peak heights of alleles in PLACE-SSCP analysis. To enable large-scale characterization of candidate SNPs with minimal human intervention, we have developed a laboratory information management system, dbQSNP, which works on the Unix server and can be accessed from client machines. This system designs experimental projects, analyses data of SSCP and sequencing, and stores relevant information. Genotyping results of SSCP and sequencing are checked for consistency to avoid errors coming from data handling. Software dedicated for capillary array-based SSCP analysis has been developed and integrated in this system. It performs SNP typing and SNP allele frequency determination in the following steps. 1) Peak detection in raw data of SSCP electrophoresis, and normalization of inter-capillary variation of mobility. 2) Allele definition of polymorphic STS and SNP identification. 3) Allele frequency determination of pooled samples. Allele frequencies are calculated automatically using data of pooled DNA and heterozygote. SNPs that cause subtle mobility shift can be quantified semi-automatically using a tool for peak separation. Hundreds of SNP data have already been processed using this database system.

**Primate Genome Evolution Analyses with BAC End Sequences.** *S. Zhao*<sup>1</sup>, *E. Eichler*<sup>2</sup>, *A. Shvartsbeyn*<sup>1</sup>, *M. Krol*<sup>1</sup>, *J. Cheng*<sup>3</sup>. 1) Dept of Mamalian Genomics, Inst for Genomic Research, Rockville, MD; 2) Dept of Genetics, Case Western Reserve University, Cleveland, OH; 3) Lawrence Berkeley National Laboratory, Berkeley, CA.

Random and targeted BAC clones of primates human, chimpanzee, baboon and lemur were electronically placed onto the human genome through the paired BAC end sequence matches. By examining the sequence conservations and long range variations, we analyzed human evolution of 60 million years. BACs that were targeted to hypervariable duplicated regions showed extensive structural rearrangement between closely related species as well as potential structural polymorphism within the human population. In contrast, unique region of the human genome were remarkably stable over evolutionary time and very little evidence of structural polymorphism (inversion, deletion or translocation) could be found within these regions. Duplicated regions present problems for human genome assembling, our data are therefore likely to facilitate the finishing of the human genome.

**Novel Human Ancient Sequence Genes Defined by *D. Melanogaster* Homology.** G.A. Bruns<sup>1,2</sup>, R.E. Eisenman<sup>1</sup>. 1) Genetics Division, Children's Hosp, Boston, MA; 2) Dept of Pediatrics, Harvard Medical School, Boston, MA.

More than 45 novel human genes that have significant homology in the *D. Melanogaster* genome but do not recognize known proteins or motifs have been identified by cross phylum database search. These genes are members of a small subset of loci that encode ancient conserved sequences of as yet unknown function. They define new protein families, some of which can be expected to have fundamental roles in cellular processes. BLAST searches of the *D. Melanogaster* EST database were carried out using human unique Unigene sequences as bait. Any high scoring retrieve that recognized a homolog of known function, a member of a function family, or an identified motif was excluded. The E values of the novel human ancient sequence genes ranged from  $e^{-131}$  to  $e^{-010}$  with the vast majority having scores of at least  $e^{-015}$ . In numerous cases, the extensive homology extended throughout the protein. Nearly all of these retrieves recognized the corresponding *C. elegans* homolog and many, the *S. cerevisiae* relative as well. RNA expression data for these novel human ancient sequence genes is being compiled.

In this study, human ESTs and mRNAs were used as bait for cross phylum database search. From our prior analysis of chromosome 22 and 21, a rich source of additional novel human ancient conserved sequence loci may be the category of "predicted genes" from the genomic sequence.

**Development of a system for parallel genotyping of human P450 single nucleotide polymorphisms: The CodeLink P450 Bioarray.** *J.D. Winick, A. Zilbergleyt, M.R. Bonner, B. Chui, P. Gwynne, K.R. Luehrsen, M. Gaskin, G.L. Kiser, T. Peters, T. Peck, T.J. Raich, Y. Jia, A. Gandhi, M. Hernandez, T. Perez, E. Pestova, D. Fermin, T.-C. Cheah, J. Gu, C.T. Yamashiro.* Motorola BioChip Systems, Tempe, AZ.

The Cytochrome P450 gene family is involved in drug metabolism and mutations in family members are indicative of an individual's response to many drugs. P450 genotyping will form the basis of pharmacogenomic studies in drug metabolism that relate to: systemic drug metabolism, inter-individual variability in drug metabolism, explanation of some specific toxic effects of drugs and explanation of multi-drug interactions. The CodeLink P450 Bioarray assay system developed at Motorola Life Sciences can detect 75 single nucleotide polymorphisms (SNPs) for 7 human P450 genes (CYP1A1, 1A2, 1B1, 2D6, 2C19, 2E1, and 3A4). The CodeLink SNP platform utilizes DNA polymerase-mediated, allele-specific extension of anchored oligonucleotide probes attached to a three-dimensional activated matrix. Each pair of allele-specific oligonucleotide probes is complementary at their 3' ends to the polymorphic position of a SNP. The P450 gene family is highly homologous between sub-family members at the nucleotide level and the inter-gene homology represents a challenge to developing a genotyping assay. PCR primer pairs were designed to amplify P450 DNA gene fragments without the amplification of highly related pseudogenes or homologous family member sequences. The CodeLink P450 Bioarray assay is a three step process: first, gene-specific target DNA is amplified by PCR from genomic DNA using CodeLink P450 Primer plates; second, the Allele Specific Extension assay is performed on the chip with CodeLink SNP genotyping reagents; and third, genotype data analysis is performed on scanned chip images using our proprietary CodeLink SNP calling algorithm. The data is tabulated and exported to a database using CodeLink Analysis software. Using DNA samples derived from known tissue culture lines the CodeLink P450 Bioarrays perform with a 99% call rate and a 99% accuracy as confirmed by conventional sequencing methods.

**Ultra-High Throughput Genotyping Platform using Arrayed Glass Plates and Primer Extension.** *P.A. Bell, X. Zhao, E. Huang, C. Emerich, R. Zhao, J.C. Wu, S. Varde, D. Kahn, K.E. Scott, C.A. Gelfand, M. Ruben, F. Jones, K. Chokshi, P. Farnsworth, V. Bogdanov, R. Kopla, F. Modica, A. Recber, A. Yasseen, J. Tarantino, M. Kochersperger, A. Huang, F.S. Kuo, M. Pohl, G. Schnerr, M. Boyce-Jacino.* Dept Biochemical Development, Orchid Biosciences, Inc, Princeton, NJ.

The success of the human genome project has increased interest in single nucleotide polymorphism (SNP) genotyping as the key measure of genetic diversity. Assays designed to score these polymorphisms are playing an increasing role in genome mapping and pharmacogenetic studies. To date, genome wide scans, and studies involving thousands of SNPs and patient samples have been hampered by the lack of a system that can perform genotyping with the needed throughput, reliability and cost. To address this need, we have developed an automated, ultra-high throughput system; SNPstream UHT™, which uses SNP-IT™, our proprietary single base extension technology. Assay design for the UHT system is performed with Orchids AutoPrimer™ software. The system uses microarrays manufactured in a 384-well format using a novel glass-bottomed plate and disulfide coupling chemistry. >10-plex PCR and genotyping are performed in homogeneous reactions, and assay results are read by direct fluorescence with our two-color SNPscope™ imager. We present here results of UHT genotyping on a panel of SNPs derived from The SNP Consortium database and human DNA samples from the Coriell repository. Using the UHT system, we have demonstrated the feasibility of analyzing 100,000 SNP genotypes per 8hr day.

**A high throughput SSCP method for large-scale analysis of SNPs/mutations using capillary-array electrophoresis system.** *Y. Kukita<sup>1,2</sup>, K. Higasa<sup>1</sup>, S. Baba<sup>1</sup>, M. Nakamura<sup>1</sup>, S. Manago<sup>1</sup>, A. Suzuki<sup>1</sup>, T. Tahira<sup>1</sup>, K. Hayashi<sup>1</sup>.* 1) Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; 2) Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Characterization of human genome diversity is a major task of the genome project at the post-sequencing stage, and the technique to detect SNPs/mutations with low running cost is awaited for, by many researchers in medical science, who are ready to analyze their samples to elucidate genetic background of various human traits. We previously developed a cost-efficient mutation/SNP detection method, PLACE-SSCP, which is a combination of post-amplification fluorescent labeling of PCR products and automated capillary-electrophoretic SSCP analysis. We present here, a high throughput version of PLACE-SSCP, which uses commercially available capillary-array electrophoresis system, i.e., MegaBACE1000, ABI3100 and ABI3700. The main obstacle in the development had been the unavailability of sieving matrix and absence of the software suitable for the analysis. We first synthesized two different polymers, which were polyacrylamide for coated capillaries used by MegaBACE1000 and polydimethylacrylamide for uncoated capillaries used by ABI machines, respectively. The SSCP software, which can process raw electropherograms from instruments, was also developed in our laboratory. All instruments using the polymers described above showed high reproducibility between capillaries after alignment of sample peaks by internal markers, when analyzed by the software described above. We observed improved separation of alleles in buffers of lower pH and higher ion concentration. Analyses of more than 100 SNPs showed that the detection rate of polymorphic alleles was more than 90% in one electrophoretic condition. We conclude that the capillary-array based PLACE-SSCP is suitable for large-scale analysis of SNP/mutation detection because of its high sensitivity, high throughput and low running cost.



**SNPStream® MT, a microsphere-based, Primer Extension System for the Analysis of Single Nucleotide Polymorphisms.** *T.M. Mulcahy, M.A. Kunkel, D. Greeny, R. Liberchuk, A. Camisa, J. Studebaker, C.A. Gelfand, P.A. Bell, M. Boyce-Jacino.* Dept Development, Orchid Bio Sciences, Princeton, NJ.

The increasing interest in single nucleotide polymorphism (SNP) genotyping and the pharmacogenomic implications of correlating SNPs to phenotypes have resulted in the need for simple, highly efficient and accurate genotyping methods. Orchid BioSciences has developed SNPstream MT, an adaptation of Orchids SNP-IT primer extension genotyping technology to Luminexs microsphere-based assay platform. Validation of this system for multiplex genotyping was performed using 48 human DNA samples on a broad SNP panel. The SNP panel was chosen to include all possible biallelic SNPs (i.e. AG, TC, TA, TG, GC, AC) and to yield polymorphic data. The results of these experiments demonstrate that SNPstream MT is a highly efficient genotyping system that facilitates the analysis of multiplexed solution phase SNP-IT assays with a high degree of accuracy. The advantages of this microsphere-based system will be described along with details of multiplex genotyping and data on the accuracy and reproducibility of the results that have been achieved with the system.

**Development of the first Center for Genomic Medicine in Mexico.** *G. Soberon*<sup>1</sup>, *JP. Laclette*<sup>2</sup>, *A. Serrano-Perez-Grovas*<sup>3</sup>, *R. Tapia-Conyer*<sup>4</sup>, *JC. Valdes-Olmedo*<sup>1</sup>, *G. Jimenez-Sanchez*<sup>5,1</sup>. 1) Mexican Health Foundation (FUNSALUD); 2) National Autonomous University of Mexico (UNAM); 3) National Council for Science & Technology (CONACYT); 4) Secretary of Health (SSA), Mexico; 5) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD, USA.

The Human Genome Project made evident the molecular bases of human individuality. Population analyses have identified polymorphisms associated with susceptibility and resistance to common multifactorial diseases. This information along with development of new genomic technologies has led to Genomic Medicine (GM), a more predictive and preventive medicine, with important social and financial implications. Mexico has over 50 different ethnic groups that contribute to the unique genomic makeup of the Mexican population. The first polymorphisms related to common diseases in our population have been identified. These findings, along with Mexico's long tradition of excellence in medicine and genetics, have provided bases for the first Mexican Center for Genomic Medicine (CEMEGEN). This national reference center for GM, will develop basic and clinical investigation contributing to the knowledge of the human genome, with particular reference to several Latin American populations. The unique horizontal structure of the CEMEGEN will stimulate continuous interactions with other academic centers in Mexico and the rest of the world. CEMEGEN will generate human resources and establish strong links with industry to capitalize new information through services and products for Latin America. In preparation for the foundation of CEMEGEN, on October 3, 2000, our initiative was funded through an official agreement signed by the heads of the National Autonomous University of Mexico, the Secretary of Health, the National Council for Research and Technology and the Mexican Health Foundation, to develop a feasibility study. This analysis has been completed and provides strong data to support scientific, technological, social, academic, political and economical feasibility to develop the CEMEGEN. This new institution will ensure high quality scientific research and production of human and material resources to develop GM in Mexico.

**Bench-top genotyping using a 96 strip-well primer extension SNP-IT™ assay.** *B.C. Sundaresan, M. Xu, K. Davis-Fleischer, M. Phillips, P.A. Bell, M. Boyce-Jacino.* Application Research, Orchid Biosciences, Inc., Princeton, NJ.

With the increasing availability of genomic DNA sequences, single nucleotide polymorphisms (SNPs) are fast becoming a powerful tool for genetic research, gene mapping and pharmacogenetics. While there are a number of tools available for SNP scoring, all of them require time consuming optimization and can require expensive instrumentation. We have adapted single base primer extension (SNP-IT) technology into an easy to use 96 well format that specifically overcomes these issues. The SNPware™ 96 Kit provides a cost effective, flexible and high quality solution to low or moderate volume SNP genotyping. DNA primers for the assay are designed using Orchids proprietary primer design software, through Orchids web site Autoprimer™.com. The DNA region that includes the SNP of interest is PCR amplified using one un-modified and one phosphorothioate modified primer. The addition of phosphorothioate groups to one of the primers enables single strand generation during the process. Single strand target DNA is hybridized to a SNP-IT oligonucleotide immobilized on the surface of a 96-well microtiter plate. Following hybridization, single base primer extension occurs by the addition of DNA polymerase and labeled terminators. The incorporated base is detected by an ELISA based colorimetric detection scheme. Results are readable by eye, or with a standard microplate reader. In a demonstration of the approach, 60 different SNPs with a genome-wide distribution were selected from The SNP Consortium database for testing with 20 Coriell DNA samples. SNPware 96 data analysis software, developed at Orchid, was used to visualize the data and facilitate genotype calling. This study demonstrates the utility of the SNPware 96 kit to perform genotyping studies without requiring costly instrumentation.

**TempliPhi™, F29 DNA polymerase based Rolling Circle Amplification of templates for DNA sequencing.** *T.L. Giesler, Y.C. Cai, J.W. Farchaus, J.R. Nelson.* Genomics Reagents, Amersham Pharmacia Biotech, Piscataway, NJ.

We have developed a DNA amplification kit that employs rolling circle amplification to generate high quality templates for DNA sequencing reactions. The TempliPhi™ kit takes advantage of the fact that cloned DNA is typically located in circular vectors. These are readily replicated *in vitro* using F29 DNA polymerase. This single subunit, proofreading DNA polymerase utilizes excellent processivity and strand displacement properties to generate multiple, tandem double stranded copies of the input circular DNA. High amplification levels (2-3 mg) can be obtained in a short time (4 hours) using random hexamer primers to initiate rolling circle amplification on very small amounts of input circular DNA. Input templates can be as little as 0.01 ng of purified plasmid DNA, a single bacterial colony or a small aliquot of a saturated overnight culture. After an initial denaturation step (3 minutes at 95°C), the amplification reaction proceeds at 30°C. F29 DNA polymerase can initiate synthesis from many primers on each circle, simultaneously advancing multiple replication forks around the circle in a highly processive fashion, generating as much as 10<sup>7</sup>-fold amplification of the input circular DNA. Once completed, the product can be added directly into sequencing reactions. This novel workflow can be completed in less than 5 hours with only two simple manipulations and eliminates the need for liquid cell cultures and template purification prior to DNA sequencing. Results obtained using this isothermal method of amplification to prepare sequencing templates from clones in M13, plasmid and cosmid vectors are presented. \*Address all correspondence to J.R. Nelson. E-mail: john.nelson@am.apbiotech.com.

**Isolation of the full-length cDNAs for "predicted genes" and finding "novel genes" on human chromosome 21.** *J. Kudoh, K. Shibuya, J. Wang, I. Obayashi, M. Tatsuyama, M. Takahashi, K. Kawasaki, A. Shintani, T. Sasaki, A. Shimizu, S. Asakawa, S. Minoshima, N. Shimizu.* Department of Molecular Biology, Keio University School of Medicine, Tokyo, Japan.

The chromosome 21 mapping and sequencing consortium including us finished 33.83-Mb genomic sequencing of chromosome 21 and identified 225 genes including 98 predicted genes (Nature, 405:311-319, 2000). We are now performing full-length cDNA isolation of "predicted genes" which were identified by sequence similarities to known genes and/or spliced EST matches and/or exon prediction programs. Putative exon sequences were used to amplify cDNA fragments by PCR using cDNAs from various human tissues. We identified nearly full-length cDNAs for predicted genes including DSCR6, C21orf20, C21orf21, C21orf22, ANKRD3 (ankyrin repeat domain 3), ZNF298, C21orf25, ZNF295, TMPRSS3 (transmembrane protease, serine 3), TSGA2 (testis specific gene 2), and SNF1LK (SNF1-like kinase), and a cluster of keratin-associated protein (KAP) genes on 21q22.3. Among these, TMPRSS3 was found to be responsible for the autosomal recessive nonsyndromic deafness DFNB8/DFNB10 (Nature Genet. 27:59-63, 2001). Furthermore, we recently identified another cluster of KAP genes on 21q22.11. Thus chromosome 21 may contain more than 40 KAP genes including pseudogenes in addition to the previously identified 225 genes. Because of difficulties in finding novel KAP genes, which are short single-exon genes and are hardly found by any exon prediction programs, we estimate that there may be hundreds of KAP genes in the human genome. The detailed transcript map and sequence information are available through our Web site (<http://www.dmb.med.keio.ac.jp>). These information and resources will be useful for identification of disease related genes, especially those responsible for a particular phenotype of Down syndrome or bipolar affective disorder, and structural and functional analysis of each of these genes.

**Pyrophosphorolysis activatable oligonucleotides have a high sequence specificity throughout their lengths.** *Q. Liu, S.S. Sommer.* Dept Molecular Genetics, City of Hope Natl Medical Ctr, Duarte, CA.

Pyrophosphorolysis activated polymerization (PAP) was initially developed to enhance the specificity of allele-specific PCR for detection of known mutations in the presence of a great excess of wild type allele. The high specificity of PAP derives from the serial coupling of pyrophosphorolysis-mediated activation of a pyrophosphorolysis activatable oligonucleotide (P\*) followed by extension of the activated oligonucleotide. Herein, we demonstrate that AmpliTaqFS or ThermoSequenase DNA polymerases greatly improve the efficiency of PAP, making it a practical technique for detection of rare mutations. We also show that pyrophosphorolysis activatable oligonucleotides (P\*) have the novel and unexpected property of high sensitive to mismatches throughout at least the sixteen 3' terminal nucleotides. PAP constitutes a technology platform of potential utility whenever high specificity is required along the length of an oligonucleotide, e.g. rapid microarray-based scanning for unknown mutations or analysis of chromatin structure.

Program Nr: 1633 from the 2001 ASHG Annual Meeting

**Human chromosome 21 updated annotation and additional resources at RIKEN GSC.** *T.D. Taylor.* RIKEN Genomic Sciences Center, Yokohama, Japan.

Chromosome 21 is the smallest human chromosome and is a model for physical mapping and sequencing of the entire human genome. Three copies of this autosome cause Down syndrome, the most frequent genetic disorder associated with significant mental retardation. The complete sequence of chromosome 21 provides a unique resource for understanding the molecular pathophysiology of Down syndrome, as well as all other monogenic and complex disorders that map to this chromosome, including Alzheimer's disease, leukemia, autoimmune disease, epilepsy and manic-depressive psychosis. It also stands as a structural framework from which the complete molecular architecture of the chromosome can be determined.

In an on-going effort to improve the quality of the sequence, several minor changes have been incorporated and are now available from our web site (<http://hgp.gsc.riken.go.jp/>) and in the public databases. The annotation data has been significantly enhanced, with more types of analyses having been performed. Of note is the inclusion of new genes, SNP information from clone overlaps and other sources, cross-species comparison analysis, additional gene-finding predictions, and homology information. Tables with summary information about the chromosome and statistical findings (i.e., frequency of alternatively-spliced exons) are also available on our web site. Several corrections have also been made to the annotation, thanks in part to feedback from the community. Of the remaining gaps, one clone gap has been partially filled, and all the sequencing gaps have now been sized.

We will continue to make updates, to make more views of the data available, to layer additional analyses and information on top of the existing data such as gene confirmation/expression data, and to include any additional genes or regulatory elements that are subsequently identified.

Program Nr: 1634 from the 2001 ASHG Annual Meeting

**From SNPs to Medical Utility.** *A. Braun.* Genomics, Sequenom, Inc., San Diego, CA.

The completion and availability of the entire human genome sequence is enabling for the discovery of genes and gene products involved in human complex disorders. The successful identification of these genes is dependent on available sample sets, a high-throughput scoring technology and an underlying scientific hypothesis on how to use the samples and the technology. Sequenom has developed a chip-based mass spectrometry approach for the analysis of single nucleotide polymorphisms (SNPs) the most abundant genetic variations, which is complemented by a fully automated SNP assay development procedure and the rapid assessment of allele frequencies in sample pools. This allows the cost effective testing of virtually all gene-based genetic variations and the association of the results with a variety of different phenotypes. We are currently developing the worlds most comprehensive set of reagents to test for SNPs. A scientific strategy using this reagent set for elucidating the major genetic factors involved in human diseases will be presented.



**Identification of disease associated genes and tumour class prediction by genome wide microarray-based DNA methylation scanning.**

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Class prediction is of crucial importance for most therapeutic decisions in cancer. Recent studies have shown that classification of cancers can be achieved by mRNA expression monitoring. However, due to difficulties with handling mRNA samples, expression analysis is not widely used for large-scale analyses or clinical settings. Here we present a novel, more robust approach to classify human cancers based on analysis of characteristic DNA methylation patterns. Information on methylation status was obtained for many sites in parallel using a DNA-based microarray. Methylation patterns were then presented to a learning algorithm to perform class predication. In addition, hierarchical clustering methods were used to show that class discovery is also possible. Our results demonstrate that analysis of methylation patterns combined with supervised and unsupervised learning techniques constitutes a powerful tool to classify human cancers.

**A real-time PCR assay for detection of disease susceptibility in an hereditary oxyradical overload condition.** *J. Fuchs, M. Podda.* Dept. of Dermatology, University of Frankfurt, Germany.

Hemochromatosis gene (HFE) linked hereditary hemochromatosis (HH), an autosomal recessive disorder, is one of the most common inherited diseases among individuals of Northern European ancestry. Two sites of point mutations in the HFE gene-C282Y and H63D-are associated with greater than 90% of HH cases. HH is a classic example of chronic iron toxicity with production of oxidative stress. In homozygotes and compound heterozygotes the massive iron overload may cause dysfunction of the liver, heart, and pancreas, finally leading to hepatic cirrhosis, liver cancer, diabetes and cardiac insufficiency among other sequelae. 10% of the Northern European population appears to be heterozygous for the hemochromatosis gene and most of these persons have elevated body iron levels, but are otherwise asymptomatic. Although it is commonly believed that the high frequency of heterozygotes may be related to a selective advantage in the past because of their slightly increased iron stores, in present times these individuals may be less in favor because of chronic co-exposure to oxidizing environmental pollutants leading to supra-threshold oxidative stress. Thus heterozygotes may be more likely to develop pathologic sequelae such as atherosclerosis, carcinogenesis and aging. If this hypothesis is true, HH heterozygotes may constitute an antioxidant responsive subpopulation that may benefit from preventive strategies and antioxidant supplementation therapy. We describe a rapid and reliable mutation analysis method for detection of the HFE mutations C282Y and H63D in mutant homozygous, wild-type, and heterozygous samples. This robust real time PCR assay is ideal for the clinical setting and for mass screening programs and will be employed for studies investigating the association of hemochromatosis heterozygosity and disease susceptibility.

**Performance Assessment of the Serial Analysis of Gene Expression (I-SAGE™) Molecular Biology Kit.** *P.N. Gilles<sup>1</sup>, Y. Zhang<sup>1</sup>, C. Stalder<sup>1</sup>, S. Roach<sup>1</sup>, A. Waters<sup>1</sup>, S.Y. Wong<sup>1</sup>, D. Mehta<sup>1</sup>, B. Cook<sup>2</sup>, S. Madden<sup>2</sup>, D. Brown<sup>1</sup>, M. Gleeson<sup>1</sup>.* 1) Genomics, Invitrogen Corp, Carlsbad, CA; 2) Genzyme Molecular Oncology, Framingham, MA.

SAGE™ or Serial Analysis of Gene Expression (Velculescu et al., Science 270, 484, 1995) is an established method for generating quantitative genome-wide expression profiles. The technique is based on the acquisition of 10-bp sequence tags, which identify each unique mRNA transcript, independent of prior sequence information. Ligations of two tags (ditag) serve as short uniform sized templates for PCR amplification with minimal bias and sensitivity for low copy transcripts. The SAGE library is constructed by the serial concatenation of ditags into elongated molecules which allows for the rapid sequencing and digital enumeration of 20 to 60 transcripts per sequencer lane. Importantly, SAGE experimental results are directly comparable to existing libraries in the public SAGEmap expression database (SAGEmap:<http://ncbi.nlm.nih.gov/>). A traditional molecular biology kit (I-SAGE™) has recently been developed and is commercially available from Invitrogen Corp. In this study, the I-SAGE™ Kit was evaluated for the representative expression of three housekeeping genes, elongation factor -1a, b-actin and glyceraldehyde 3-phosphate dehydrogenase, in the human lung carcinoma A549 cell line. The quantified expression of these housekeeping genes was evaluated in libraries generated from the manufactured I-SAGE™ Kit and compared to Beta test lots and to libraries generated with conventional SAGE methodology by an independent laboratory. The above analysis validated that the libraries generated using the I-SAGE™ Kit reflect conventional SAGE expression profiles and can be used to perform comparative analysis with other databases. The I-SAGE™ Kit facilitates the generation of complex and comprehensive expression profiles (>100,000 tags), which will contribute to the identification, cloning and characterization of genes involved in normal physiologic and pathologic states.

Program Nr: 1638 from the 2001 ASHG Annual Meeting

**Rapid analysis of biochemical modifications in DNA fragments - Visualisation of phosphorylation and cytosine deamination in single 15 minute analyses.** *M. Daniels<sup>1</sup>, M. Dickman<sup>2</sup>, D. Hornby<sup>2</sup>*. 1) Transgenomic Ltd, Crewe Hall, Crewe, Cheshire, UK; 2) Transgenomic Research Laboratory, Krebs Institute, University of Sheffield, Sheffield, UK.

The analysis of biochemical modifications in nucleic acids is often difficult, requiring multiple steps including enzymatic or chemical reactions, the need for fluorescent or radioactive labels, and the use of agarose or polyacrylamide gel electrophoresis with subsequent blotting and/or autoradiography. In addition, the interpretation of data from these analyses can be demanding in order to discern the presence of artefacts and correctly identify reaction products. A single analysis may reasonably be expected to run over two to three days. In order to overcome these limitations we have utilised the WAVE® Nucleic Acid Fragment Analysis System as an analysis platform to visualize a phosphorylation reaction catalysed by polynucleotide kinase and a cytosine deamination repair reaction catalysed by uracil DNA glycosylase. These analyses could equally be used to assay the respective enzyme activities, or to assay the quality of DNA with respect to phosphorylation or cytosine deamination. The WAVE® System readily resolved both phosphorylated from non-phosphorylated oligonucleotides and uracil containing from apyridinic oligonucleotides. The analysis required less than 15 minutes per sample and no radioactivity was required.

**Cross species hybridization of human microarrays to study diseases and mechanisms of drug action. L.M.**

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DNA microarray technology provides the opportunity to look at the genome wide expression of disease processes and mechanism of drug action. Utilizing this technology to examine animal models of disease as well as studies that cannot be done in cell culture or in humans would be very useful. Microarrays have been developed for human, mouse, rat, yeast, and the fruit fly. The purpose of this study is to evaluate whether samples of other species can be analyzed by cross-hybridization to human DNA microarrays. We hybridized samples from various tissues of dog, cow, and monkey to Affymetrix human microarrays which interrogate ~ 6,000-12,000 genes and ESTs. Because of sequence differences between species, we anticipated that cross-hybridization to human arrays would result in a decreased hybridization of some of the perfect match probes and a increased hybridization to some of the mismatch probes; consequently there would be a decrease in the number of genes called "present" by the Affymetrix algorithm. Using standard protocols, we found that the average number of genes called "present" on the cross-hybridized arrays varied between ~ 11%; in dog, ~ 12% in cow, and ~ 35% in monkey, compared to ~ 40% in human. We observe that when hybridization intensity is significant, the pattern of expression for a specific gene across the 20 probe pairs of a set is remarkably similar between species. This indicates that data obtained on probes sets not called "present" can also be used by taking into account the non-specificity of the probe set across gene family members. Furthermore, clustering of microarray data, independent of the Affymetrix "present" or "absent" call, from various human and monkey tissues indicates that gene expression profiles generated by cross-hybridization to human arrays still provides useful information such as tissue-specific gene expression patterns. We conclude that, while cross-hybridization to human microarray is not as robust as same species hybridization, it is possible and offers an opportunity to generate very valuable information on gene expression profiles.

**Optimization of an External Standard for the Normalization of Affymetrix GeneChip® Array Data.** K.J.

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Expression profiling using nucleic acid hybridization-based methods have become prevalent in medical and biotechnological research and development, diagnostic testing and drug development. It allows the researcher to screen thousands of genes on a single chip thus making this a time efficient technique. The reliability and utility of nucleic acid hybridization-based methods depends on accurate and reliable methods for accounting for variations between analyses. For example, variations in hybridization conditions, label intensity, reading and detector efficiency, sample concentration and quality, background effects, and imaging processing effects each contribute to hybridization signal heterogeneity. One of the challenges with the use of microarrays is identifying the optimal approach for normalizing one chip to another. One approach currently utilized is the use of global scaling for chip normalization. However, this analysis has significant limitations when comparing chips with different number of expressed genes. Here, we introduce the optimization strategy that utilizes 11 external spiked-in cRNAs for the use of generating the best standard curve to normalize chips to each other. We performed *in silico* and wet lab experiments to identify, from the 40,000,000 possible combinations of the 11 cRNAs, the best assignments for the individual concentrations of the external cRNAs. The generated standard curve is then used to convert gene expression intensity values into frequency numbers. We have demonstrated sensitivity of the external cRNAs down to 0.5 pM, 95% of the time. This external normalization has been validated on numerous GeneChip® arrays, like human, rat, mouse, yeast and *Arabidopsis* with  $R^2$  of approximately 0.985. We showed that external normalization has improved the data by allowing for comparison between genes on different chip types, higher accuracy in fold change analysis, as well as more powerful multivariate analysis.

**Technologies for generating, normalizing and subtracting cDNA libraries from small quantities of mRNA.** S. Bashiardes, Y. Korshunova, R. Tidwell, D. Hawkins, M. Lovett. Genetics, Washington University, St. Louis, MO.

An increasing number of expression-based studies focus upon generating accurate profiles from small amounts of RNA. We have modified several cDNA techniques to derive a method that allows for the construction of a representative cDNA library from as little as 5ng of mRNA without overt skewing in sequence representation. First strand cDNA is synthesized on a solid matrix and a modified SMART oligo is added to this. The cDNA is then PCR amplified through 15 cycles using nested primers and is cloned by ligation-independent methods. We have used this method to construct ten different cDNA libraries from microdissected tissues. In this method first stand cDNAs are retained on the solid matrix and can be used to derive targets for gene chip hybridizations by linear run-off amplifications. The method thus allows one to build cDNA libraries and conduct expression profiles from one small RNA sample. We have also developed new modifications to cDNA normalization and subtraction methods that are compatible with these libraries. These involve using PCR amplified and biotinylated inserts in a hybridization with very pure single stranded libraries of cDNAs. We have employed these methods in the generation of 8 normalized and two subtracted cDNA libraries. In a separate project we are also developing methods for building full length cDNA libraries. These are not immediately applicable to small amounts of RNA, but have great utility in extending parts of genes or ESTs. We have developed a method (double capture) that targets both the 5' and 3' ends of the mRNA:cDNA duplex following first strand synthesis (through the 5' CAP and 3' polyA tail). The 5' capture is achieved by binding to a synthetic aptamer. This yields approximately 10% of the synthesized first strand cDNA. The 3' capture is achieved using a specially designed Digoxigenin labelled capture oligo. Using a combination of this oligo and RNase treatment, we select against cDNA molecules synthesized through mispriming events. cDNAs synthesized from bone fide poly(A) tails are bound to anti-Dig magnetic beads. The resulting cDNAs are cloned and then evaluated by DNA sequence analysis.

**PCR device for highly parallel sample preparation for multiplex SNP genotyping platforms.** *M.T. Cronin, E.S. Mansfield, D. Dutta, M. Pho, J. Wu, M. Liu, B. Chen, A. Nguyen, D. Albagli, G. Bogdan, B. Silver, M. Tang, R. Anderson.* Applied Genomics, ACLARA Biosciences, Inc, Mountain View, CA.

The focus of human genomic research is rapidly shifting away from acquiring primary sequence data to cataloging and characterizing sequence variability. Mendelian and complex genetic disorder mapping is accelerating as dense genetic maps of common sequence polymorphisms evolve. The resulting sharp increase in demand for high throughput genotyping is being addressed with highly parallel analysis technologies such as hybridization arrays, bead arrays, mass spectrometry and capillary array electrophoresis. However, sample preparation remains a bottleneck effectively limiting access to these parallel readout methods. Generally, widely dispersed informative sequence polymorphisms, including SNPs and STRs are analyzed as pooled products from multiple single-plex PCR reactions, limited multiplex PCR reactions, or higher levels of multiplex PCR that require extensive assay development. This is to prevent primer interaction or allelic dropouts that complicate analysis and may lead to typing errors or decrease the yield of genotyping results. Amplification and labeling steps done in low multiplicity tend to be inefficient since it adds sample processing steps and leads to substantial reagent waste. We have developed a novel LabCard™ device to simultaneously amplify 960 PCR reactions using standard thermocyclers. More consistent representation of allelic products and better signal-to-noise were observed when the multiplex PCR was carried out in the prototype LabCard device than conventional multiplex tube reactions. Samples prepared using this device are compatible with a wide range of multiplex genotyping methods. Representative analytical data from primer extension based genotyping of informative SNPs in drug metabolizing enzyme genes using SNaPshot™ biochemistry and ABI 3100 analysis will be presented, as will additional data from samples prepared using LabCard devices for multiplex target amplification followed by primer extension labeling analyzed by array hybridization or MALDI-TOF detection.



**Perioperative Genomic Profiles With the Invader® Flap Endonuclease System.** *R.W. Kwiatkowski<sup>1</sup>, R. Selzer<sup>2</sup>, D. Green<sup>2</sup>, E. Rasmussen<sup>1</sup>, K. Hogan<sup>2</sup>.* 1) Third Wave Technologies, Madison, WI; 2) University of Wisconsin-Madison, WI.

Over 40,000,000 patients undergo anesthesia and surgery per year in North America. Despite profound heritable differences in response to potent drugs and stress of surgery, genetic factors are at present unaccounted for in advance. Here we compare the analytical validity of the novel Invader genotyping system with conventional PCR-based RFLP and sequencing methods for a panel of alleles, each with specific and well-established clinical utility. Genomic templates (blood, cheek swab) from 213 patients having outpatient, peripheral vascular, neurosurgical or solid organ transplant procedures were assayed for 690 genotypes using both PCR/RFLP and Invader assay techniques for 31 pathologic alleles in the BChE, RYR1, CACNA1S, CYP2D6, FV, FII, MTHFR, MTR, MTRR, CBS, TNF and genes. Overall concordance was 99.6% (687/690). The three discordant samples all appeared in the BChE locus (G1615A). The Invader assay reproducibly called all three heterozygote while PCR/RFLP results revealed wild type status, which was confirmed by PCR-sequencing. Invader assays are typically run directly on genomic DNA without prior target amplification using PCR. In an effort to resolve the discrepancies, the Invader assay was also run on PCR product for the three discordant samples. In that case the Invader assay results agreed with the wild type call made by the PCR based assays. The reason for the difference when this locus was tested directly on genomic DNA is unknown. DNA preparation or storage is suspect, the three discordant samples had been prepared using non-standard methods and stored over 10 years. We conclude that the Invader genotyping system is capable of high precision in the detection of SNPs, insertions, and deletions in both non-coding and coding regions underlying traits of clinical significance that may be genetically heterogeneous. Advantages in operator efficiency, cost, and safety, coupled with avoidance of PCR-based cross-contamination and mis-incorporation point to important advantages of Invader-based methods in genotyping many millions of patients for hundreds of alleles in the perioperative interval.

**Making DNA microarrays optimization and comparison of various DNA immobilization strategies.** *S. Kmoch<sup>1,2,3</sup>, L. Ondrová<sup>1,2</sup>, M. Nová<sup>1</sup>, M. Jebková<sup>1,2</sup>, M. Hřebíček<sup>1,2,3</sup>*. 1) Inst Inherited Metabolic Dis, Charles Univ 1st Sch Medicine, Prague 2, Czech Republic; 2) Center for Integrated Genomics, Prague; 3) GeneAge Technologies, Prague.

Efficient and uniform immobilization of target DNA molecules onto a glass surface is one of the critical steps in making DNA microarrays and is central to the quality of the resulting data. We have adopted and compared immobilization of amino-modified DNA onto a poly-L-lysine and 3-glycidoxypropyltrimethoxysilane-modified glass surfaces, disulphide-modified DNA onto a 3-mercaptopropyltrimethoxysilane-modified glass surface and a disulphide-modified DNA covalently cross-linked to 3-mercaptopropyltrimethoxysilane onto unmodified glass surface. Individual chemistries were tested with Cy5 and Cy3 labeled PCR products or Cy5 labeled oligonucleotides. Target DNA was spotted by GeneSurfer arrayer (GeneAge Technologies) and individual reaction steps were followed by IVL laser scanner (Genomic Solution). Our experience showed that published immobilization protocols were usable only after careful optimization. Poly-L-lysine chemistry is useful for binding of amino-modified and even of non-modified DNA. However, blocking procedure limits its application only for hybridization experiment. Disulphide chemistry is applicable for both hybridization and primer extension experiments. Procedure is fast, effective and cheap what makes it preferable for high-throughput array production.

Program Nr: 1645 from the 2001 ASHG Annual Meeting

**Detection of Cytokine Induction in Cell Lysates Using a Biplex mRNA Invader® Assay.** *M.C. Olson, S.M. Olson, W. Iszczyszyn, P.S. Eis, T. Takova, M.L. Curtis, L. Chehak, B.T. Argue, R.W. Kwiatkowski.* Third Wave Technologies, Madison, WI.

The Invader technology is a flexible, isothermal, easy to use method that can accurately quantitate mRNA without target amplification. Low variability (3-10% coefficient of variation) provides accurate quantitation of less than two-fold changes in mRNA levels. A biplex FRET-based detection format enables simultaneous quantitation of expression from two genes within the same sample. One of these genes can be an invariant housekeeping gene that is used as the internal standard. Normalizing the signals from the gene of interest with the internal standard provides accurate results and obviates the need for replicate samples. A simple and rapid cell lysate sample preparation method can be used with the mRNA Invader assay. The combined features of biplex detection and easy sample preparation make this assay readily adaptable for use in high-throughput applications. Results will be presented demonstrating biplex detection of cytokine and housekeeping gene expression levels with automated 96- and 384-well microplate formats.

**A multiplex solution for SNP interrogation using fifth-dye labeled short size standard and single-tube primer extension reaction.** *S.S. Kuo, G. Ayanoglu, D. Wei, M. Johnson, D. Bishop, A.B. Tomaney, P. Dong.* Applied Biosystems, Foster City, CA.

A variety of methods may be used to characterize and to screen single nucleotide polymorphisms. Analysis platforms include microarray scanning, real-time PCR analysis (TaqMan), and MALDI-TOF. In addition, electrophoresis-based techniques include OLA analysis, dideoxy sequencing, and single-nucleotide primer extension (SNE). SNaPshot is a single-tube SNE reaction designed for elucidation of individual loci within known sequence contexts for the purpose of SNP screening and validation.

The completed reaction identifies one nucleotide located 3' relative to the primer site. We have reformulated our SNaPshot reagent mix to enable robust multiplex SNP interrogation against multiple templates in varying amounts. The resulting multiple products can then be analyzed by electrophoresis in the presence of a size standard, labeled with a 5th dye. Evaluations on ABI Prism Models 310, 3100, and 3700 have been successful.

We describe the development of a 5th-dye labeled short size standard for analyzing small fragments on fluorescent detection systems. This standard contains 9 fragments ranging from 15 to 120 nucleotides. During evaluation on various capillary electrophoresis platforms, we are able to achieve excellent precision and curve-fitting. The fifth dye is spectrally well-resolved from other dyes. The 5th-dye size standard is designed in particular to enable automated data analysis in methods for SNP detection. In our poster, we will demonstrate its utility in SNE assays. With the combination of different primer lengths and four-color sample chemistry, the potential for multiplexing SNP loci exists for high-throughput genotyping with minimal optimization.

**GeneJumper<sup>TM</sup>-oriV Transposon: A valuable tool for retrofitting BACs/PACs with an amplifiable origin of replication in genome sequencing.** *U. Matrubutham<sup>1</sup>, J. Liu<sup>1</sup>, D.M. Brown<sup>1</sup>, W. Szybalski<sup>2</sup>, M.A. Gleeson<sup>1</sup>.* 1) Gene Discovery, R&D, Invitrogen Corporation, Carlsbad, CA; 2) McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, WI.

Invitrogen's GeneJumper<sup>TM</sup> transposon is an artificial mini-Mu transposon used in *in vitro* transposition reaction to randomly insert primer-binding sites into target DNA. The transposon enables rapid generation of DNA templates for bi-directional sequencing without the need for shotgun cloning or primer walking of large plasmids such as BACs and PACs. We have subcloned the DNA element of the origin of replication oriV, from plasmid RK2, into the GeneJumper<sup>TM</sup> transposon (called GeneJumper<sup>TM</sup>-oriV) in order to retrofit BACs/PACs with this ori via *in vitro* transposition. This ori becomes amplifiable in the presence of a copy-up mutant of the replication initiation protein TrfA. We have validated the GeneJumper<sup>TM</sup>-oriV transposon for efficient retrofitting of BACs, increased plasmid yield upon amplification (2 to 6 ug ml<sup>-1</sup>), and stable maintenance of large plasmid upon prolonged amplification. We have also shown the applicability of the transposon for high-throughput sequencing. GeneJumper<sup>TM</sup>-oriV transposon is available from Invitrogen Corporation, Carlsbad, CA in a kit format, which consists of reagents for *in vitro* transposition, the suitable *E. coli* competent cells for oriV amplification and the primers for bi-directional sequencing.

**Application of a DNA microarray-based comparative genomic hybridization (Array-CGH) method to the study on the genetic effects of Atomic bomb radiation.** *N. Takahashi<sup>1</sup>, K. Sasaki<sup>1</sup>, N. Tsuyama<sup>1</sup>, M. Kodaira<sup>1</sup>, M. Itoh<sup>1</sup>, I. Danjo<sup>1</sup>, S. Kyoizumi<sup>2</sup>, N. Niikawa<sup>3</sup>, A. Fujiyama<sup>4</sup>.* 1) Lab Biochem Genet/Dept Genet, RERF, Hiroshima, Japan; 2) Dept Radiobiol, RERF, Hiroshima, Japan; 3) Nagasaki Univ Sch Med, Nagasaki, Japan; 4) Nat Inst Genet, Mishima, Japan.

For over 50 years, the Foundation has conducted a study on the effects of A-bomb radiation on human germ cells. For the study at the genome-level, it is essential to efficiently collect a large volume of genetic information from a sufficient number of subjects. It is also important to accurately detect a gene deletion which occurs on an autosome (in most cases, radiation-induced mutations appear to be about 1 Mb deletion type), since the deletion appears as a change from 2 allele copies to a single copy. Comparative genomic hybridization (CGH) was developed for genome-wide analysis of DNA copy number in a single experiment. However, conventional CGH limits detection of events involving small deletion (of less than 20 Mb). On the other hand, there are reports that Array CGH can detect single-copy decrease and increase from normal diploidy, and have higher resolution power than conventional method. Thus, the Array CGH is considered to be an effective method for our study. We therefore tested the effectiveness in an experimental system. Cosmid DNA clones containing DNA fragments from either autosomes or X chromosomes, and Pac DNA containing the fragments, which were deleted in Prader-Willi syndrome (PWS) patients, were immobilized on a glass. The DNA from females labeled with Cy5 was used as internal control, and the DNAs of females, males, or PWS patients were separately labeled with Cy3 and used as a test sample. A mixture of labeled DNAs was hybridized and quantified. We demonstrated that female-DNAs contain 2 copies, and male-DNAs contain 1 copy from the fluorescence ratio on the spots of X chromosome's clones. We also found that there was a single copy decrease in DNAs from the PWS patients on the spots of the corresponding clone spots. These results indicate that the Array CGH can detect changes in the copy number of specific loci in genome-DNA. Thus, this method should prove most useful for our study.

**SNP Detection on Solid Surface Using the Invader® Assay.** *P. Wilkins Stevens<sup>1</sup>, J. Hall<sup>2</sup>, V. Lyamichev<sup>2</sup>, B. Neri<sup>2</sup>, D. Kelso<sup>1</sup>.* 1) Biomedical Engineering, Northwestern University, Evanston, IL; 2) Research & Development, Third Wave Technologies, Inc., Madison, WI.

We have demonstrated that the invasive cleavage reaction can be performed on a solid phase. The solution-phase invasive cleavage assay, a signal-amplification method capable of distinguishing nucleic acids that differ by only a single base, is already an effective tool for many genomic applications such as scoring single nucleotide polymorphisms, tracking genotypes of viruses and microorganisms, and monitoring gene expression. The method positions two overlapping oligonucleotides, the probe and upstream oligonucleotide, on the target nucleic acid to create a complex recognized and cleaved by a structure-specific 5-prime nuclease. In the solid-phase experiments, both the probe and upstream oligonucleotide were attached to the surface of microparticles, which served as the solid-phase platform. The probe, configured with a 5-prime terminal dabcyl molecule and an internal fluorescein, was fluorescence-quenched until target-directed cleavage released the dabcyl quencher. Targets based on the ApoE 158 Cys and Arg alleles were tested with particles coated with Cys- or Arg-allele probes, and target-specific probe cleavage was documented. The particle-bound Cys probes were enzymatically cleaved only when the target was based on the Cys allele, while the particle-based Arg probes were cleaved only when the target corresponded to the Arg allele. Effective cleavage of the probe oligonucleotide occurred when probe and upstream oligonucleotides were positioned away from the particle surface via long tethers; a number of different tether lengths were tested.

Three different methods for reading fluorescent signal from the particles are being investigated: real-time monitoring of the fluorescence of a suspension of particles with a fluorometer, measuring the fluorescence of individual particles on a fluorescence-activated cell scanner, and reading the fluorescence of particles immobilized on a microscope slide.

**Whole genome amplification with a highly frequent degenerated oligo pair (HFDOP).** *K. Zhang<sup>1</sup>, L. Jin<sup>2</sup>.* 1) Human Genetics Center, UTHHSC-SPH, Houston, TX; 2) Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

Whole genome amplification has its wide application in a variety of genetic analysis wherever the amount of DNA available is limited. Several techniques have been proposed for the whole genome amplification, including inter-IRS PCR, IRS-Bubble PCR, tagged PCR and DOP PCR. It has been shown that DOP PCR not only produce a good coverage, but also is technically simple and robust. However, based on the recently published human genome draft sequence, our analysis shows that approximately 20 percent of the human genome do not have a primer pair in proper orientation within the range of 5Kb, which is the upper size limit for normal PCR amplification, and hence is not accessible for DOP PCR amplification. Here we report a pair of primers for whole genome amplification designed with a novel strategy. Firstly, we established an oligo distribution database based on the human genome draft sequence for the oligos between 6 mers to 12 mers. Highly frequent probabilistic "contigs" were assembled from those 10mers that have more than 10,000 copies in the haploid genome, and totally twenty seven primers were designed based on four selected contigs. Secondly, the priming capability and the genome coverage of these primers and their combinations was tested both electronically and experimentally, then a degenerated primer pair, term as highly frequent degenerated oligo pair (HFDOP), was selected for whole genome amplification. Electronic PCR indicates that the inaccessible region of the human genome can be reduced 3 percent, which is also supported by gel electrophoresis that shows much more bands for PCR with our primer pair than that for DOP PCR. Finally, we labeled the PCR products with fluorescent dyes, hybridized them with a human cDNA expression array containing 2764 genes. By comparing the number of spots, which have pixel distribution significantly different from that of the local background, we found that the coverage of the PCR product with HFDOP is approximately 43% higher than that with DOP. In summary, HFDOP PCR is a robust and simple whole genome amplification method with much higher genome coverage than other WGA methods available.



**An efficient application of MALDI-TOF/MS coupled with micorarray for detection of microsatellite**

**polymorphism.** *Y. Yoshikawa*<sup>1</sup>, *K. Nakajima*<sup>4</sup>, *N. Kimura*<sup>1</sup>, *M. Gonda*<sup>2</sup>, *K. Okamoto*<sup>4</sup>, *M. Ota*<sup>3</sup>, *G. Tamiya*<sup>4</sup>, *H. Inoko*<sup>4</sup>.  
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The analysis of microsatellite polymorphisms mainly depends on the electrophoretic separation of fluorescently labeled PCR products. There is an increasing demand for genotyping method that can provide large amount of genetic information in a time-efficient and cost-effective manner.

To address this request, we have been developed an alternative method by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS). MALDI-TOF/MS offers unprecedented speed for analysis with excellent accuracy in measuring DNA fragment size. However, the conditions for DNA detection using MALDI-TOF/MS has not been considered, especially when a large DNA fragment is analyzed. In order to optimize the conditions for large DNA fragment, we first examined matrices, small organic molecule, which is a key part of mass spectral method. We have screened 147 compounds as potential matrices, and we find that several of these compounds were useful for the preparation and analysis of samples. Among them, the best results were obtained with 2,4-dihydroxyacetophenone. With this matrix, we successfully detected 100- and 102- nucleotide samples with extremely high-resolution that sufficient to distinguish single-nucleotide difference. Next, we examined carbodiimide-coated glass plate instead of expensive stainless-steel plate that commonly used to MALDI-TOF/MS. As a result, we clearly detected PCR products of 200 bp on this glass plate. Moreover, microarray-based hybridization assay is verified using the glass plate. Conditions for hybridization were optimized, and we were able to detect the DNA fragment hybridized on the glass plate using MALDI-TOF/MS without any non-specific signals.

These results indicate that MALDI-TOF/MS coupled with microarray technology is now emerging as a powerful approach of high-throughput and cost-effective alternative to gel electrophoresis for precise determination of polymorphic microsatellites.

**New tools to study proteins involved in disease: FSHD as paradigm.** *S. van Koningsbruggen*<sup>1</sup>, *H. de Haard*<sup>2</sup>, *R.W. Dirks*<sup>1</sup>, *J.T. den Dunnen*<sup>1</sup>, *G.W. Padberg*<sup>3</sup>, *G.J. van Ommen*<sup>1</sup>, *C.T. Verrrips*<sup>2</sup>, *R.R. Frants*<sup>1</sup>, *S.M. van der Maarel*<sup>2</sup>. 1) Center for Human & Clinical Genetics and Dept. of Molecular Cell Biology, LUMC Leiden, Leiden, Netherlands; 2) Unilever Research, Vlaardingen, Netherlands; 3) Dept. of Neurology, UMCN Nijmegen, Nijmegen, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is caused by a complex genetic rearrangement. Likely, partial deletion of the subtelomeric D4Z4 repeat on 4qter causes the transcriptional deregulation of nearby genes by a position effect variegation-like mechanism. Consequently, there are no obvious structural mutations within the FSHD candidate genes, complicating their positive identification.

Next to expression profiling of candidate genes, we have embarked on protein profiling employing llama-derived phage-display single (heavy) chain antibody fragments. *Camelidae* carry apart from a conventional antibody repertoire, a unique repertoire of heavy-chain antibodies that only consist of two heavy chains and are devoid of light chains. Using this antibody repertoire as source for phage-display libraries circumvents the in vitro combination of heavy and light chains, one of the major drawbacks of conventional phage-display libraries. Moreover, these antibodies have unique properties regarding affinity and stability. Current strategies are directed in optimizing high-throughput selection and screening protocols and design of phage-display vectors allowing uniform arraying, visualization, and intracellular expression. Due to their small size (14kDa) and high affinities, autofluorescent heavy-chain antibody fragments will be eminently suited for a variety of applications.

As proof of principle, we successfully generated immune-libraries raised against cocktails of proteins and whole human skeletal muscle homogenate. We selected high-affinity antibody fragments against FRG1P, the gene product of one of the candidate genes for FSHD. These antibody fragments perform well in a series of immunological techniques: for example, co-localization was observed for EGFP-tagged FRG1P and selected heavy-chain antibody fragments.

**Full-length cDNA cloning of tissue specific expressed sequence tags.** *Z. Xu<sup>1</sup>, L. Jia<sup>2</sup>, K. Eng<sup>1</sup>, C. Xia<sup>1</sup>, D. Jablons<sup>1</sup>.*  
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One of the ultimate goals of high through-put EST sequencing is the development of resources for study on functional genomics of different tissues. A large number of tissue specific expressed sequence tags (EST) have been isolated. Because of short sequences for ESTs, it is important to isolate full-length cDNA clones for functional analysis of the tissue-specific ESTs. We have developed a library-free method for rapid full-length cDNA cloning. The method involves ligating an anchor sequence to 3 ends of the first strand cDNA using T4 DNA ligase after reverse transcriptase reaction. An oligo-nucleotide primer specific to tissue related ESTs was biotinylated and hybridized with the first strand cDNA pool. EST-specific first strand cDNA was captured by streptavidin-coated magnetic beads and eluted. The purified EST-specific first strand cDNA was then amplified using high fidelity thermal stable DNA polymerase and cloned into plasmid vectors. We have successfully cloned full-length cDNAs for several known human genes including p53 (2.5 kb), transferrin receptor (5 kb) and CFTR (6.2 kb) using this approach. Four bone tissue specific ESTs (453, 467, 509, 835) were tested using this method. Full-length cDNA fragments were successfully amplified and cloned from three of the four ESTs tested and the complete inserts were under sequence analysis. The results demonstrate that this library-free method is more efficient for cloning full-length cDNAs than conventional method and should be useful for generating full-length clones for tissue specific ESTs. (Supported in part by NIH grant R21CA85172).

**Generation of high specificity cRNA Probes from 1 ug of total RNA for Hybridization onto DNA Microarray Glass Slides.** *J. Tajbakhsh, S.F. Nelson.* Human Genetics, UCLA, Los Angeles, CA.

DNA microarray technology is a powerful method to simultaneously monitor the activity of thousands of genes thus allowing the parallel analysis of gene expression changes in a given biological context. Since its introduction as a high-throughput method DNA-microarrays have been used to address questions in a variety of biomedical fields. Typical gene expression analyses using glass arrays require relatively large quantities of total RNA (up to 100 ug) or poly A+ RNA (5ug). However in a number of application fields the amount of starting material is very low. For instance, samples prepared by microdissection of small tissue regions or collected from patient biopsies or embryonic material often contain only a few cells and therefore yield relatively little RNA. To date several approaches have been reported to circumvent probe limitation either by an enzymatic amplification of the poly A+ message, involving PCR, or by a post-hybridization signal amplification step. In order to rule out the possibility of introducing biases via non-linear amplifications, we have developed a simple, reproducible, and representative method which allows us to perform gene expression profiling on DNA microarrays from 1ug of total RNA. In this approach double-stranded cDNA is synthesized from total RNA including a promoter for the T7 RNA polymerase on its upper strand, followed by one round of T7 based in vitro transcription (IVT) in which modified UTP molecules (aminoallyl-UTP or Cy3/Cy5-UTP) can be incorporated into nascent antisense RNA (aRNA) products. In this way enough RNA can be amplified (up to 600 fold) to sensitively probe a spotted array while maintaining the relative abundance of mRNA species. We have experienced that the combination of RNA amplification and cRNA labeling in one step is time saving and cost effective and therefore offers an attractive way to generate hybridization probes for a large number of microarray analyses with limiting samples.

**Single Label, Single Reaction, Single Nucleotide Polymorphism Genotyping.** *S.A. Varde, M.A. Kunkel, C.A. Gelfand, M.T. Boyce-Jacino.* Assay Research, Orchid BioSciences, Inc., Princeton, NJ.

For correlating molecular markers (single nucleotide polymorphisms or SNPs) with disease states, either to diagnose a disease or to identify a drug target, it is imperative to have assays that are robust and simple to use. Orchid has developed a bi-allelic SNP-IT<sup>TM</sup> single nucleotide primer extension technology that will facilitate a single SNP genotyping reaction that uses only a single color. This SNP-IT assay involves using multiplex PCR as a template and is carried out in solution phase as a multiplex reaction in a single well using a single label on each of the allelic terminating nucleotides for detection. The analysis can be performed on various platforms and its simplicity enables it to be easily automated. Furthermore, the requirement of only one labeled terminator makes the assay cost-effective. The same assay scheme can be applied to our more traditional two-label assay with redundant data that makes possible even higher confidence genotype scoring, and enabling all four base identities to be genotyped in multiplexed reactions with only two labels. Assay results using a variety of platforms using both the single- and dual-label variations of this SNP-IT assay are presented.

**The 1.4 Mb CMT1A duplication/HNPP deletion genomic region reveals unique genome architectural features and provides insights into the recent evolution of new genes.** *J.L. Badano<sup>1</sup>, K. Inoue<sup>1</sup>, K. Dewar<sup>2</sup>, N. Katsanis<sup>1</sup>, L.T. Reiter<sup>1</sup>, E.S. Lander<sup>2</sup>, K.L. Devon<sup>2</sup>, D.W. Wyman<sup>2</sup>, B. Birren<sup>2</sup>, J.R. Lupski<sup>1</sup>.* 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Whitehead Institute for Biomedical Research, Cambridge, MA.

Duplication and deletion of the 1.4 Mb region in 17p12 that is delimited by two 24 Kb low copy number repeats (CMT1A-REPs) represent frequent genomic rearrangements resulting in two common inherited peripheral neuropathies, Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsy (HNPP). A gene within the 1.4 Mb region, *PMP22*, is responsible for these disorders through a gene dosage effect in the heterozygous duplication or deletion. However, the genomic structure of the 1.4 Mb region, including other genes contained within the rearranged genomic segment, remains essentially uncharacterized. To delineate genomic structural features, investigate higher order genomic architecture, and identify genes in this region we constructed PAC/BAC contigs and determined the complete nucleotide sequence. This genomic segment contains 1,421,129 bp of DNA. A low copy number repeat was identified, with one copy inside and two copies outside the 1.4 Mb region. Comparison between physical and genetic maps revealed a striking difference in recombination rates between the sexes with a lower recombination frequency in males (0.67 cM/Mb) versus females (5.5 cM/Mb). Hypothetically, this low recombination frequency in males may enable a chromosomal misalignment at proximal and distal CMT1A-REPs and promote unequal crossing over, which occurs 10 times more frequently in male meiosis. In addition to three previously described genes, five new and 13 predicted genes were identified. Analyses of the genomic region adjacent to proximal CMT1A-REP indicated an evolutionary mechanism for the formation of proximal CMT1A-REP and the creation of novel genes by DNA rearrangement during primate speciation. In addition, using this finished genomic sequence a set of 15-STR markers that can detect >99% of CMT1A duplication was developed for potential diagnostic use.

**Smith-Magenis syndrome repeat gene clusters - structure, evolution, and breakpoints of genomic rearrangements in human and gorilla.** *P. Stankiewicz*<sup>1</sup>, *S.-S. Park*<sup>1</sup>, *W. Bi*<sup>1</sup>, *L. Potocki*<sup>1</sup>, *J. Lehoczyk*<sup>3</sup>, *K. Dewar*<sup>3</sup>, *B. Birren*<sup>3</sup>, *J.R. Lupski*<sup>1,2</sup>. 1) Depts. of Molecular & Human Genetics; 2) Pediatrics, Baylor College of Medicine, Houston TX; 3) Whitehead Institute for Biomedical Research/MIT Center for Genome Research, Cambridge, MA.

Over 90% of SMS patients are deleted for an identical ~4Mb 17p11.2 genomic region, whereas in the remainder both smaller and larger sized deletions were identified. Our studies demonstrated the presence of three large, complex low-copy repeat gene clusters: proximal SMS-REP (progenitor, ~260Kb) is in the same orientation as distal SMS-REP (~190Kb), whereas middle SMS-REP is inverted. These architectural features enable substrates for non-allelic homologous recombination resulting in both deletions and reciprocal duplications of the same region within 17p11.2. This chromosome segment is also associated with breakpoints of the *idic(17)(p11)* chromosome (CML, PNETs) and with the breakpoint of an evolutionary chromosome *t(4;19)* in gorilla. These observations suggest that it is susceptible to mitotic and meiotic rearrangements and that higher order genomic architecture involving low-copy repeats plays a significant role in the genome evolution during primate speciation. To delineate the genomic structure and evolution of SMS-REPs we constructed a complete BAC contig, are determining the genomic sequence, and performed FISH and Southern hybridization studies in several primate species. We identified that SMS-REPs are absent in mice and were duplicated after the divergence of New World Monkeys from pre-monkeys, >40Mya. In addition to a strong signal on 17p11.2, BACs containing SMS-REP showed in FISH studies weaker signals on 17q11.2, 17q12q21, and 17q24. BLAST analysis revealed that SMS-REPs shares ~25Kb fragments of 99% homology with NF1-REPs. To further investigate the relevance of SMS-REPs structures to chromosome rearrangements, we analyzed the breakpoints of unusual sized deletions in SMS patients. The SMS critical region was narrowed to ~1Mb. All 5 patients with a smaller sized deletions have their proximal breakpoints mapping within the middle SMS-REP and their distal breakpoints within or adjacent to distal SMS-REP.

**Identification of genes involved in susceptibility to bipolar affective disorder using GeneChips.** *L.J. Adams, P.R. Schofield.* Neurobiology, Garvan Institute, Sydney, Australia.

Bipolar affective disorder (BD) is a major psychiatric condition characterized by severe mood disturbances. BD is treated by anti-manic drugs such as lithium and anti-epileptic/anti-manic drugs such as valproate, although we still know little about how these drugs exert their therapeutic mood stabilising effects. We are using the mouse as a model to investigate the expression of genes that are modulated by anti-manic drugs through the use of microarray studies. Our premise is that since the currently used drugs take several days to effect therapeutic benefit, they must cause changes in gene expression in the brain as an essential prerequisite to the onset of clinical effectiveness. Genes found to be commonly modulated by more than one of the anti-manic drugs are candidates that may have a role in the etiology of BD. We have validated treatment doses in mice for both lithium and valproate to achieve the equivalent of human therapeutic serum levels of these drugs. We have obtained brains from mice treated for 7 days with either valproate (400 mg/kg/day, n = 10); lithium (340 mg/kg/day), separated into those with high (0.8-1.2 mM; n = 10) or medium (0.4-0.7 mM; n = 10) serum levels of lithium; or saline controls (n = 10). Our initial experiments were undertaken using Affymetrix Murine U74A GeneChips, each containing approximately 12,000 transcripts, and mice (n = 5) with high lithium serum levels (meanSD = 0.940.03 mM) or saline controls (n = 5). Analysis of these GeneChips identified 20 transcripts expressed at least two-fold more, and two genes expressed at least two-fold less in the lithium treated animals compared to the controls. These represent members of different gene families, including protein kinases, transcription proteins, and integral membrane proteins. These studies may help to elucidate the underlying cellular events that ultimately lead to the onset of bipolar disorder, and the biological pathways that are involved in disease pathogenesis.



**Construction of a microarray on 5q31-q33 region array to identify genes controlling resistance or susceptibility to parasitic diseases as schistosomiasis and malaria.** *C. Chevillard<sup>1</sup>, N. Haribou<sup>1</sup>, R. Tagett<sup>2</sup>, B. Lorient<sup>2</sup>, R. Houlgatte<sup>2</sup>, C. NGuyen<sup>2</sup>, A. Dessein<sup>1</sup>.* 1) INSERM U399, faculty of medicine, Marseille, 13385, france; 2) INSERM U136, parc scientifique de luminy, case 906, Marseille, 13288, france.

Schistosomiasis affects 200 million people and is a major public health problem in many southern countries. Our group performed studies on the causes of high infections in an endemic area of Brazil. Certain subjects appeared to be predisposed to high infections whereas others always exhibited low infection in spite of high exposure. This suggested that host-specific factors were important in the control of infection. Using segregation and linkage analysis, it was shown that there was strong evidence for the control of infection by a major locus (SM1) which was located in the 5q31-q33 region which contains a number of gene that encode cytokines that play an important role in the regulation of immune response against parasites. Immunological studies performed on the same population showed that SM1 control is linked to the differentiation of the T helper cells into Th1 or Th2 lymphocytes. Furthermore, it was also reported that blood parasitemie in *Plasmodium falciparum* are controlled by a locus located in the same region. This region is containing a large number of genes. In order to facilitate the analysis of this locus we have developed a systematic expression level analysis of all the coding regions of the 5q31-q33 interval by microarray technology. The target will be a I.M.A.G.E. clone set (approximately 800) arrayed on nylon support. The probe will be labeled with 33P. Indeed combination of nylon array with 33P labeled radioactive probes provides 100 fold better sensitivity, making it possible to perform expression profiling experiments using submicrogram amounts of unamplified total RNA from small biological samples. Generation of a 5q31-q33 region specific array will be essential for study of several others diseases as asthma familial hypereosinophilia. The results obtained in the analysis of mRNA expression in subjects with schistosomiasis will be presented.

**Functional analysis of promoter SNPs.** *B. Hoogendoorn, K. Smith, S. Coleman, C. Guy, P.R. Buckland, M.C. O'Donovan.* Dept Psychological Medicine, Univ Wales Col Medicine, Cardiff, Wales.

Single nucleotide polymorphisms (SNPs) in the regulatory regions of genes may affect transcription by altering binding or recognition sites for transcription factors or RNA polymerase. Consequent variation in gene expression may be implicated in inherited psychiatric disorders. We have developed a rapid and simple method for detecting promoter activity of putative gene promoters and for comparing the effect of promoter region polymorphic variants on the expression of genes. To date, we have selected 1050 putative gene promoters identified from a variety of publicly available databases. To date, we have designed PCR primers for 630 of these promoters. The primer pairs spanned the DNA sequence 500bp immediately upstream of, and including, the start of transcription. PCR conditions were optimised for 400 of the 630 promoters, which were subsequently amplified from DNA of 16 unrelated subjects. Amplimers were screened for heteroduplex formation by DHPLC. To date, 207 out of 400 promoters were found to be polymorphic. SNPs were confirmed and characterised by sequencing. Allelic pairs of promoters were cloned into a modified pGL3 luciferase expression T-vector, and transfected into 3 cultured cell lines (HEK293T, TE671, JEG3) before measuring levels of luciferase by luminometry. By utilising 96 well plate technology and an internal control plasmid expressing secreted alkaline phosphatase, each of these allele pairs can be tested for relative promoter activity in each of three cell lines. Significant differences in activity were detected between alleles in many of the pairs assayed. This work is ongoing and the relevant numbers are likely to change considerably.

**Complete transcript map of the Wolf-Hirschhorn syndrome critical region (WHSCR) by comparative sequence analysis between man and mouse, expression analysis and functional studies.** *N. Pfarr*<sup>1,2</sup>, *S. Ende*<sup>2</sup>, *S. Schlickum*<sup>2</sup>, *C. Steglich*<sup>2</sup>, *M. Oswald*<sup>1</sup>, *B.U. Zabel*<sup>1</sup>, *J.-H. Bebermeier*<sup>2</sup>, *A. Winterpacht*<sup>2</sup>. 1) Childrens Hospital, University of Mainz, Mainz, Germany; 2) Institute of Human Genetics, University of Hamburg, Hamburg, Germany.

Wolf-Hirschhorn syndrome (WHS) is a complex and variable malformation syndrome resulting from the absence of the distal segment of one chromosome 4 short arm. Clinical and cytogenetic data indicate that WHS is a contiguous gene syndrome with an undefined number of genes contributing to the phenotype. The WHS critical region (WHSCR) has been confined to a 165 kb gene rich region on chromosomal subband 4p16.3. From clinical data (Rauch et al., 2001) and from mouse experiments (Naf et al., 2001) it is known that deletion of the WHSCR alone results in mild WHS features. Nevertheless, deletion of this region seems to be necessary (but not sufficient) for the complete WHS phenotype in man and mouse. Our studies aim at the identification of all genes and regulatory regions in the WHSCR (including flanking regions) as well as the functional characterization of the gene products. We and others have previously identified novel genes in the WHSCR and flanking region. Here, we report the reinvestigation of the WHSCR using comparative sequencing of the corresponding chromosomal region in the mouse genome (chromosome 5) and interspecies sequence comparison to identify evolutionary conserved and therefore functionally important sequences. The study was accompanied by detailed expression analysis of the resulting transcripts and by functional studies of selected genes. On the basis of these data we present a novel, complete transcript map of the Wolf-Hirschhorn syndrome critical region in man and mouse. Besides the known genes LETM1, WHSC1, WHSC2 and POL4P we identified additional putative transcribed sequences which may represent alternatively spliced exons and/or additional genes. One of these transcripts shows specific expression in several adult and fetal organs affected in WHS patients. Further activities will concentrate on the functional analysis of the transcribed sequences and on conserved non-coding regions.

**Identification of human genes specifically expressed in retina.** *S. Schultz*<sup>1</sup>, *T. Leveillard*<sup>2</sup>, *J. Sahel*<sup>2</sup>, *C. Lavedan*<sup>1</sup>. 1) Pharmacogenetics, Novartis, Gaithersburg, MD; 2) INSERM/ULP/HUS EMI 99-18, Strasbourg France.

Retinal degenerative diseases affect millions of people all over the world. Unfortunately, there are few effective treatments for retinal degenerative diseases, such as Retinis Pigmentosa and Age Related Macular Degeneration. Most retinal degenerative diseases are inherited. Several genes such as RHO and PDE6B have been shown to be mutated in some patients. The identification of other genes specifically expressed in human retina may provide new clues in understanding the development of these diseases. It may also aid in designing more specific and effective treatments. The purpose of this work was to identify genes specifically expressed in the human retina and more precisely, genes expressed in the periphery containing rod photoreceptors and genes expressed in the macula containing cone photoreceptors. Retina samples were obtained from two male individuals. From each retina, the periphery and macula were separated and RNA was extracted. The 4 samples were individually hybridized to an oligonucleotide microarray containing ~12,000 probe sets. Retina specific genes were found by comparing gene expression profiles obtained from these 4 retina samples to profiles of more than 50 different normal adult and fetal tissue samples. Comparisons of profiles were performed using our in-house Novartis Pharmacogenetics Network (NPGN) database. The comparison of the two macula samples with the two periphery samples allowed us to determine in which type of retinal cells (rod or cone) these genes were expressed. The gene expression profiles were also analyzed using GeneSpring software to identify clusters of co-regulated genes. The identification of rod and cone specific genes, combined with mapping information of disease loci, is providing a new list of candidate genes potentially mutated in patients with retinal degenerative diseases. Furthermore, clusters of genes co-expressed in different regions of the retina are providing a greater understanding of the pathways involved in the development and function of the human retina. These results should help in initiating new experiments designed toward the discovery of better therapeutics for retinal diseases.

**Validation of SNPs in asthma candidate genes on chromosome 2q from public data bases using pooled DNA samples and the MALDI TOF technique.** *T. Immervoll, H. Gohlke, M. Wjst.* Inst. of Epidemiology, GSF Research Ctr, Neuherberg, Bavaria, Germany.

During asthma inflammation many mediators are released and involved in tissue damage. A key element in the inflammatory response is the prompt production of proinflammatory cytokine IL-1 $\beta$ . Exogenous administration of IL-1 $\beta$  creates airway responsiveness. On the other hand IL-1Ra has anti-inflammatory properties. Therefore the imbalance between these proinflammatory and inhibitory cytokines may be an important determinant of asthma. These genes as well as the corresponding receptors have all been mapped to the same chromosomal region 2q12-21, where linkage with asthma and allergy genes has been detected. We therefore looked for SNPs from public available databases located in these genes (<http://www.ncbi.nlm.nih.gov/LocusLink>). Up to now 64 of 97 SNPs in genes of the IL1 gene cluster have been analysed in 94 probands of a German population study where DNA concentration was measured with cyber green and pooled into one tube. A pool of equimolar amounts of DNA of each proband was then PCR amplified in the regions of interest and the corresponding SNPs were analysed with the MALDI TOF technique (Buetow et al., 2001). Comparing mass peaks of the two possible SNP variants we calculated the frequencies of the analysed SNPs. 24 of the 64 SNPs could not be found in our population as they all had frequencies lower than 10%. 20 SNPs showed a frequency of 10 - 20% in this population, 14 SNPs from 20 -30% and six between 30 and 50%. These SNPs are now tested in the individual proband DNA samples. First results indicate that the results of the individual and the pooled DNAs do not differ more than 5 %, which should be sufficient for the association analysis of a complex diseases like asthma. -Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, Little DP, Strausberg R, Koester H, Cantor CR, Braun A (2001) High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip- based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc Natl.Acad.Sci U.S.A* 98:581-584.

**Leaky imprinting in the Prader-Willi Syndrome region.** *C.I. Brannan, S.J. Chamberlain.* Department of Molecular Genetics and Microbiology and the Center for Mammalian Genetics, University of Florida, Gainesville, FL.

Prader-Willi syndrome (PWS) appears to be a contiguous gene syndrome resulting from mutation of two or more imprinted genes. Previously, we described a mouse model for PWS in which paternal transmission of a 35 kb PWS Imprinting Center (PWS-IC) deletion mutation resulted in complete loss of local paternal gene expression. On the (C57BL/6J x 129/Sv)F1 genetic background, these PWS mice exhibit severe growth retardation, hypotonia and neonatal lethality. Our long-term goal is to rescue these neonatal phenotypes via genetic complementation in an effort to functionally identify the causative genes in PWS. Our strategy has been to create multiple lines of transgenic mice on the FVB/NJ genetic background using bacterial artificial chromosomes (BACs) that contain either individual or groups of PWS candidate genes. We have then bred transgenic females with males harboring the PWS-IC deletion mutation and genotyped and observed the resulting offspring. In the course of these experiments, we have found that the (FVB/NJ x 129/Sv)F1 mice harboring the PWS-IC deletion mutation are able to survive to adulthood provided the majority of the wild-type sibs are removed within a few days after birth. While these surviving mice are smaller than normal, they do not get obese and both males and females have proven to be fertile. To determine why these mice survive, we assayed expression of the PWS candidate genes at birth, 1 week, 2 weeks, and 3 weeks of age. We found that expression of multiple PWS candidate genes was detectable at low levels (only detectable by RT-PCR, but not by Northern blot) by 1 week of age. Even more surprising is that based on analysis of *Snrpn* we have determined that this expression is derived from the maternal FVB/NJ allele. These results lead to two conclusions. First, it appears that certain mouse strains do not silence imprinted genes as completely as other strains. This suggests that either the machinery of imprinting or the cis-elements have diverged among strains, resulting in leaky imprinting. Second, to rescue the majority of PWS phenotypes, it appears that only low levels of expression of PWS candidate genes may be required.

**Disruption of the imprinting center on human chromosome 15q11-q13 region using the DT40 cell shuttle system.**

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Human chromosome 15q11-q13 harbors multiple imprinted genes that are involved in Prader-Willi (PWS) and Angelman (AS) syndromes, two distinct mental retardation disorders caused by paternal and maternal deficiencies, respectively. Several PWS and AS patients have been found to have microdeletions in a region of upstream of the SNRPN gene referred to as the imprinting center (IC). The IC is proposed to regulate initiation of imprint switching for all genes in a 2 Mb imprinted domain during gametogenesis. Recent studies of a rare PWS family and chimeric mice with imprinting mutations showed clearly that de novo IC deletion on the paternal chromosome results in postzygotic acquisition of a maternal epigenotype in somatic cells. Certainly the IC is therefore the region of mechanistic importance in the process of genomic imprinting of 15q11-q13. However, how the IC performs this function is unknown.

Previously, we demonstrated that the targeting experiments using the DT40 cell shuttle system demonstrate that the human *LIT1* locus can act as a negative regulator *in cis* for the coordination of imprinting at the chromosomal domain. This system can be used to define regulatory elements that confer long-range control of gene activity within chromosomal domains. Thus, we are generating modified human chromosomes carrying a targeted deletion of the PWS/AS IC using the DT40 cell shuttle system to the role of IC in maintenance of parental imprint in this region.

**Transcribed single nucleotide polymorphisms as markers for screening genomic imprinting.** *G. Zhu, R.H. Lipsky, K. Xu, L.A. Akhtar, G.L. Jenkins, D. Goldman.* Laboratory of Neurogenetics, NIAAA, NIH, Rockville, MD.

Single nucleotide polymorphisms (SNPs) in transcribed regions are ideal markers for detecting allele-specific patterns of gene expression. To facilitate rapid identification of imprinted genes and to perform large scale screening of RNA samples for quantitation of gene imprinting patterns, a method for measuring allele-specific differential expression was developed and applied to two known imprinted genes: Small nuclear ribonucleoprotein polypeptide N (SNRPN) and Insulin-like growth factor 2 receptor (IGF2R) expressed in human lymphoblast cell lines. Relative allele-specific mRNA levels were determined using a reverse transcriptase (RT)-coupled 5 nuclease fluorescence assay. Genomic imprinting was determined by pair-wise comparison of SNRPN or IGF2R allele expression in mRNA from individuals heterozygous for a coding region SNP. These expression patterns were confirmed by RT-PCR based RFLP method. The parental origin of the expressed allele was confirmed by pedigree analysis.

For SNRPN, only one allele was expressed in 11 heterozygous lymphoblastoid cell lines, consistent with complete genomic imprinting, and the origin of the expressed allele was in each case paternal, as predicted. In contrast, only one of 10 heterozygous cell lines showed mono-allelic expression (maternal) of IGF2R, the other 9 heterozygotes showed bi-allelic expression of IGF2R. These results suggest that IGF2R is polymorphically imprinted. The RT-coupled 5 nuclease fluorescence assay appears to be a reliable approach for large scale screening of imprinted genes. This approach can be extended to detect unknown functional polymorphisms in promoter regions and to determine the influence of SNPs in 5' and 3' untranslated regions on gene expression.



**LD, FRAX and sequence-based maps.** *S. Ennis*<sup>1</sup>, *A. Collins*<sup>1</sup>, *A. Murray*<sup>2</sup>, *G. Brightwell*<sup>2</sup>, *N.E. Morton*<sup>1</sup>. 1) Wessex Human Genetics Inst., Duthie Building (Mp 808), Southampton General Hospital, Southampton, Hampshire, U.K; 2) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Wiltshire, U.K.

The FRAX region at Xq27-28 is one of the most intensively studied areas of the genome by virtue of its associated syndromes. Our studies on over 7000 independent haplotypes, one of the largest collections in the world, have revealed extensive linkage disequilibrium (LD) across the region (Ennis et al, 2000). We have also demonstrated that LD may be applied to evaluate the accuracy of alternative sequence assemblies within the region. Using the  $r$  (rho) parameter as the measure of association and estimating  $M$ ,  $e$  and  $L$  in the Malecot equation, where  $E(r) = Me^{-ed}$ , we compared a sequence-based map (<http://genome.ucsc.edu/>) to our older map which was based on linkage, radiation hybrid and physical mapping [Location DataBase (LDB) [http://cedar.genetics.soton.ac.uk/public\\_html/](http://cedar.genetics.soton.ac.uk/public_html/)]. The value of  $M$  remains stable and there is no evidence against  $L=0$  for either map. Evidence against the non sequence-based map is stronger than  $c^2_1 = 28$ . The swept radius (the value at which LD falls to  $\sim .37$  of its original value) increases from 420 kb for our older LDB map to 1000 kb for the sequence-based assembly, reflecting the greater length of the new map.

Our current project is designed to extend our understanding of LD in the region. We are genotyping selected subsets of our independent chromosomes for single nucleotide polymorphisms (SNPs) covering a region of approximately 4.5 Mb from 1.5 Mb proximal to FRAXA to 1.5 Mb distal to FRAXE at a density of 1 SNP per 100kb. We employ SNPs which have been first characterised in chromosomes with a clinically interesting FRAXA repeat number. These data will be used to better determine localised recombination hot and cold spots, test map order integrity and investigate any SNP associations with the dynamic mutation which causes the fragile X syndrome.

**The mouse orthologue of the glutamate receptor-like gene *GRINL1A* maps to mouse chromosome 9.** K.S.

Wydner<sup>1</sup>, B.K. Mohan Raj<sup>2</sup>, S.W. Finkernagel<sup>3</sup>, L.J. Sciorra<sup>3</sup>, R.S. Roginski<sup>2</sup>. 1) Biology, Saint Peter's College, Jersey City, NJ; 2) Anesthesiology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ; 3) Obstetrics and Gynecology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ.

An intron capture strategy involving use of polymerase chain reaction was used to identify and map the mouse orthologue of *GRINL1A*, a human glutamate receptor-like gene. Oligonucleotides complementary to adjacent exons in the rat orthologue of *GRINL1A* were used to amplify the corresponding segment from mouse genomic DNA. Sequencing of the resulting mouse DNA fragment of approximately 2 kb revealed that the exon sequences at the ends of the amplified fragment are highly homologous (96% nucleotide identity) to both the rat orthologue and the human *GRINL1A*. A *BstXI* restriction site polymorphism within the mouse intron was used to map the mouse glutamate receptor-like gene (*Grinl1a*) to mouse chromosome 9 in a region that shares linkage conservation with human chromosome 15q21-q22, to which *GRINL1A* was recently mapped by in situ hybridization. *GRINL1A* is a functional and positional candidate gene for autism susceptibility. *Grinl1a* cosegregates with several genes that are expressed in neural tissues, including *Adam10*, *Foxb1b*, *Gnb5*, *Myo1e*, and *Tcf12*.

**Do expression patterns correlate with genetic linkage regions?** *A.M. Valdes, K. Jones, A. Schafer, D. Townley.*  
Incyte Genomics, Cambridge, England.

We have investigated whether a gene expression pattern-recognition searching tool termed Guilt -by-Association (GBA) could be used to identify genes which predispose to osteoporosis. GBA (Walker et al Genome Res 1999; 9:1198) finds transcripts with similar expression patterns by reducing expression data to a binary variable (present or absent). A gene is considered to be present if cDNA corresponding to the gene is detected in the sample from that library and absent when no cDNA for that gene is detected in the library. To determine whether two genes have significantly similar expression patterns their occurrences in a large number of cDNA libraries are examined, a contingency table is built and the probability of the table under the assumption of independent expression is computed. Using GBA, we examined the gene expression in 1176 cDNA libraries, and analyzed the transcripts that mimic the expression pattern of four genes known to affect the risk of osteoporosis: vitamin D receptor (VDR), estrogen receptor alpha (ESRA), transforming growth factor beta-1 (TGFB1) and collagen type I alpha 1 (COLIA1). 219 transcripts were found to have significantly similar patterns to one or more of the above genes. Only one gene (LMP7) had a significantly similar expression pattern to all four candidate genes. The map locations of the 219 transcripts were derived by mapping their sequences to the Santa Cruz genome assembly contigs and extracting the map positions of the contigs. Of the 219 transcripts, 29 fell within the linkage regions reported in published genome-wide linkage scans for bone mineral density (BMD; Koller et al. J Clin Endocrinol Metab 2000 85:3116; Niu et al. Hum Genet 1999 104:226). These regions are: 1q21-23,11q12-13, 5q33-35,6p11-12,2p21-24 and 13q34. To assess the relevance of this finding, the mapping location of 400 random genes was derived. Only 19 of these 400 mapped to any of the above mentioned linkage regions. The proportion of sequences falling within linkage regions derived from GBA (13.2%) is significantly higher than the one derived from random genes (4.7%) with  $p < 0.0001$ . Our results suggest that GBA can be a valuable tool in the search for genes involved in susceptibility to complex diseases.

**High-resolution human genome-wide integrated radiation hybrid and linkage maps.** *T. Matisse<sup>1</sup>, S. Sabuktagin<sup>1</sup>, E. Sulman<sup>2</sup>, E. Katz<sup>2</sup>, C. Porter<sup>3</sup>, P. White<sup>2,4</sup>.* 1) Genetics, Rutgers Univ, Piscataway, NJ; 2) Oncology, Children's Hospital of Philadelphia, PA; 3) Genome Database, Toronto, ON, Canada; 4) Pediatrics, University of Pennsylvania, Philadelphia, PA.

Even as complete genomic sequence is becoming available, high-resolution maps of the human genome continue to serve many important roles in sequence assembly, disease gene and comparative mapping, disease risk prediction, and understanding mechanisms of recombination. As part of our eGenome data integration project, we have constructed a novel set of high-resolution linkage and radiation hybrid (RH) maps, using publicly available data. Although other groups have used overlapping subsets of the same data to construct similar maps, our approaches are different, more comprehensive, and provide a unique and complementary resource. Combined with including the most comprehensive sets of markers, these unique maps can play very useful roles for the ongoing functions of linkage and RH maps and can assist in more accurate draft sequence assemblies.

We first constructed a novel set of RH maps using all publicly available markers (> 54,000) scored in the GB4 panel and obtained from the Radiation Hybrid Database (RHdb). A stringent mapping approach was employed using the MultiMap, RADMAP, and CRI-MAP computer programs. Polymorphic markers within this set were identified and given priority for placement on the map, to improve integration with our linkage maps. We then constructed novel meiotic linkage maps using all publicly available polymorphic markers (> 12,000) genotyped in the CEPH reference pedigrees and obtained from the CEPH Genotype Database, the Marshfield Center for Medical Genetics, and the CHLC. The subset of polymorphic markers present on our backbone RH maps were used as initial skeletal maps for the construction of meiotic linkage maps using procedures similar to those used for RH mapping. The average resolution (Mb/map interval) is 0.96 and 1.5 and density (markers/Mb) is 10 and 4 for the RH and linkage maps, respectively. These maps have been integrated into our eGenome database (<http://egenome.chop.edu>).

Program Nr: 1671 from the 2001 ASHG Annual Meeting

**A Higher Resolution Genetic Map.** *A. Kong, D.F. Gudbjartsson, G.M. Jonsdottir, M. Frigge, S.A Gudjonsson, J. Sainz, T.E. Thorgeirsson, B. Hallbeck, S. Sigurdardottir, J. Gulcher, K. Stefansson.* Decode Genetics, Reykjavik, Iceland.

By genotyping 146 Icelandic nuclear families with over 6000 polymorphic markers, we have constructed a genetic map based on 1257 meioses, 658 male and 659 female, or more than six times the number of meioses upon which the Marshfield map is based. In addition to providing a more accurate map for linkage analysis, the larger sample size provides substantially higher resolution for studying the variation of recombination rates across the genome, the relative recombination rates between sexes, the correlation between recombination rates and linkage disequilibrium, and the relationship between recombination rates and sequence parameters including G+C content, density of genes, density of SINEs, LINEs, LTR and DNA elements and other repeated elements. Previously unknown local patterns of recombination rates have been detected throughout the genome. We have also used the data to identify and correct some problems in the current assembly of the public sequences.

**Human physical map version 05 and discrepancies with the UCSC sequence draft assembly.** *J. Sainz, S. Gudjonsson, K. Agnarsson, O. Atladottir, U. Benitez, S. Einarsson, E. Gonzalez, O. Traustason, S. Palsson, G. Sigurdsson, D. Gudbjartsson, G. Jonsdottir, M. Frigge, A. Kong, K. Stefansson, J. Gulcher.* Decode Genetics Inc, Reykjavik, Iceland.

We have developed an automatic method to integrate data from different sources to rapidly construct genomic physical maps. We used the method to generate five consecutive versions (V01 to V05) of human genome physical maps. In version05 we integrate experimental and in silico data with data from the public domain including the working draft assembly from the 12 December 2000 freeze released by UCSC. The experimental data includes hybridization of 10,388 microsatellite markers versus the RPCI-11 BAC library. We also integrated a high resolution genetic map generated at Decode. Map V05 includes among other data, 71,670 UniGene clusters in 82,740 locations, 10,932 RefSeqs in 20,512 locations, 5,847 expressed sequences containing trinucleotide repeats with a total of 7,644 repeats, 164,669 STSs and 11,610 microsatellite polymorphic markers. Comparison among the order of markers in our map and in the UCSC sequence revealed some strong discrepancies. We are currently modifying the draft sequence assembly according to our physical and genetic maps.

**On PREDs and ORFs of human chromosome 21.** *M. Friedli, C. Henrichsen, F. Chapot, M. Guipponi, R. Lyle, A. Reymond, S. Antonarakis.* Division of Medical Genetics, Univ. of Geneva Medical School, Geneva, GE, Switzerland.

An extra copy of chromosome 21 causes Down Syndrome (DS), the major genetic cause of mental retardation, which affects approximately 1 live born child in 700. To understand the molecular pathogenesis of DS, it is necessary to identify all Human Chromosome 21 (HC21) genes. The sequence of the entire human chromosome 21 identified 151 genes, while 79 novel anonymous genes solely identified by gene prediction and/or spliced ESTs still need to be confirmed as *bona fide* transcripts. Experimental documentation of full-length transcripts is of importance since bioinformatic analyses do not always accurately predict the full genomic structure of a gene, or provide detailed information about the pattern of expression, alternative splicing and the 5 and 3 extremities of the transcript. To identify candidate genes for DS phenotypes we are systematically sequencing human ESTs mapping to chromosome 21 and murine ESTs mapping to the syntenic regions on mouse chromosomes 16, 17 and 10, and corresponding to the published PREDs and C21ORFs. This allowed to refine the gene models by combining two predicted genes in one (e.g. C21orf11 and PRED44), by splitting a predicted gene in two (e.g. PRED54), by showing that the predicted gene does not correspond to a transcript as another gene is present at this locus (e.g. PRED56), or by showing that the predicted gene correspond to a pseudogene (e.g. PRED31); the analogous gene mapping elsewhere on the human genome. The revision of the HC21 transcriptome will have implications for the rest of the genome in terms of the quality of previous annotations and the total number of the transcripts, as well as providing new candidates for genes involved in Down Syndrome.

**Dissecting the genomic patterns of methylation status by novel melting curve based methods.** *D.T. Akey, J.M. Akey, K. Zhang, L. Jin.* Environmental Health, University of Cincinnati, Cincinnati, OH.

DNA methylation is the covalent modification of cytosine to 5-methylcytosine at CpG dinucleotides and may have a myriad of functional consequences such as mediating tissue and developmental-specific gene expression and providing a mechanism to control endogenous parasitic mobile genetic elements. Moreover, aberrant regulation of DNA methylation can lead to various types of cancer and neurodegenerative diseases. The two most common methods to determine methylation status are Methylation Specific PCR (MSP) and COBRA. Both of these assays require initial modification of DNA by sodium bisulfite, which converts unmethylated cytosines to uracil. MSP relies on PCR primers specific for methylated versus unmethylated DNA whereas COBRA employs restriction enzymes to differentiate methylated and unmethylated DNA. Although these methods are powerful and conceptually straightforward, in practice MSP and COBRA are not amenable to high-throughput analyses because they are labor intensive and expensive. To address these limitations, we have adapted MSP and COBRA to a melting curve analysis (MCA) platform (which we denote as  $M^C$ MSP and  $M^C$ COBRA) that eliminates the need for gel-electrophoresis. MCA is performed by slowly heating DNA fragments in the presence of the double stranded DNA specific fluorescent dye SYBR Green I. As the sample is heated, fluorescence rapidly decreases when the melting temperature of a particular fragment is reached. Our data suggests that MCA is at least 10 times as sensitive compared to gel-electrophoresis. In addition,  $M^C$ MSP and  $M^C$ COBRA are well suited for high-throughput applications because 96 samples can be analyzed and automatically scored in under 15 minutes. Furthermore, preliminary data suggests that  $M^C$ MSP and  $M^C$ COBRA allow quantitative estimates of methylation status. We demonstrate the use of  $M^C$ MSP and  $M^C$ COBRA by assessing the methylation status in the promoter region of two important tumor suppressor genes, p16 and p15 from normal tissue and bladder tumors. In conclusion,  $M^C$ MSP and  $M^C$ COBRA allow the methylation status of CpG dinucleotides to be determined in a simple, inexpensive, and high-throughput manor.



Program Nr: 1675 from the 2001 ASHG Annual Meeting

**Rapid, High Precision Analysis of DNA and RNA Footprinting Reactions Utilising the WAVE® Nucleic Acid Fragment Analysis System.** *M. Dickman<sup>1</sup>, M. Piff<sup>1</sup>, M. Daniels<sup>2</sup>, D. Hornby<sup>1</sup>*. 1) Transgenomic Research Laboratory, Krebs Institute, University of Sheffield, Sheffield, UK; 2) Transgenomic Ltd, Crewe, UK.

The analysis of footprinting reactions is a highly involved process, normally requiring the use of a radioactive label, polyacrylamide gel electrophoresis and autoradiography. This method of analysis may take two to three days before results are available and requires skilled interpretation to avoid errors. By utilising the WAVE® Nucleic Acid Fragment Analysis System we developed an automated protocol for DNA and RNA footprinting reactions with a 15 minute run-time without the requirement for radioactive labels. Comparison of previously published analysis of an RuvA/Holliday junction complex using conventional polyacrylamide gel electrophoresis and X-ray crystallography with the WAVE® System gave predictions of a protected region extending over 13 bases, 8 bases and 9 to 10 bases, respectively. We demonstrate that the greater accuracy and precision of the WAVE® System in comparison to gels in separating the footprinting reaction products results in a more accurate prediction of the protected region. Footprinting of a hairpin ribozyme complex gave results in agreement with previously published data. This analysis is quantitative and interpretation is assisted by automatic integration of peak areas with graphical display to assist in identification of protected bases.

**Use of fluorescein-labeled probes as a quality control tool for cDNA microarrays.** *M.J. Hessner, K. Dowling, O. Kokanovic, L. Meyer, S.H. Nye, X. Wang, J. Waukau, S. Ghosh.* Pediatrics, Medical College of Wisconsin, Milwaukee, WI.

DNA microarrays enable expression analysis of thousands of genes simultaneously making them a tool of growing popularity in the genetics research community for identification of candidate genes involved in human disease. Generation of quality arrays depends on a number of steps that are difficult to measure, especially when dealing with potentially thousands of cDNA amplicons. Recent reports have identified probe quality and quantity as critical control points. We have developed a means of quality control for probe amplification, array/element morphology and post-process probe retention, and potentially normalization of inter-element variation by generation of probes with fluorescein-labeled primers. This approach eliminates the need to evaluate probe quality by gel electrophoresis as well as the need to visualize arrays with a DNA stain to evaluate probe retention and element morphology. Visualization of the entire array at any point from printing through hybridization is possible with no spectral interference when evaluating Cy3 or Cy5-labeled hybrids. The use of fluoresceinated probes for microarrays necessitates purification of amplified PCR products prior to printing in order to remove unincorporated dye-labeled oligonucleotide primers. We have evaluated high-throughput methods for probe purification and have found Promega's MagniSil system an effective, high-recovery, automatable alternative to column-based purification products. By titrating purified labeled probes, we have demonstrated that a significant percentage (>75%) of probe printed in 50% water/50% DMSO is not retained by poly-L-lysine coated glass slides. Smaller array elements and greater probe retention is observed when printing is performed in 3X SSC/1.5 M trimethylglycine as reported by Diehl et al. [NAR, 29(7):e28]. We conclude that the use of labeled probes is affordable and can greatly facilitate methods optimization and array quality control.

**Comparison of spotted cDNA arrays and Affymetrix oligonucleotide arrays: High concordance under stringent analysis parameters.** *F. Martinez-Murillo, E.P. Hoffman.* Research Center for Genetic Medicine. Center III. Children's National Medical Center. Washington, DC.

We present a systematic comparison of data obtained from the two commonly used expression profiling experimental platforms: spotted cDNA arrays, and Affymetrix oligonucleotide GeneChip arrays. Using murine tissues expected to be highly discordant in expression profiles (brain and liver), we processed aliquots of the same RNA samples for hybridization to multiple replicates of each microarray type (6 arrays with 4,600 murine gene cDNAs printed in duplicate, and 2 Affymetrix GeneChip arrays with 12,600 murine probe sets for U74A and U74B, respectively). Both experimental platforms gave highly reproducible results, with replication of experiments showing Pearson correlation coefficients of 0.96-0.98 for absolute analyses for Affymetrix arrays, and 0.95-0.99 for ratio analyses for cDNA arrays. Fold-change analysis of Affymetrix arrays (comparison analyses of brain versus liver expression profiles) showed almost as high a correlation, with  $r$  values between 0.93-0.98. In both methods the gene expression comparisons showing the greatest variability corresponded to those where one RNA sample showed expression of a specific gene near background levels and where the other showed high level expression, leading to the expected volatility of the resulting ratio. We were able to compare a total of 257 significantly differentially expressed probe sets common to both platforms. Comparison of the fold-changes for these shared genes showed that some of the most discordant results were those where the denominator approached background hybridization levels. Additional discordances were seen with saturation on specific probes and with low-level transcripts. Overall, when considering all sources of experimental error and ratio volatility, we excluded 41 outlier calls from the total 257 compared probe sets and we observed an outstanding correlation between the two methods, with  $r = 0.90$  and an  $t$ -test showing an overall  $p < 0.001$ . In conclusion, we have found that both methods are comparable and sensitive, although intrinsic properties of each system requires some user intervention in data analysis.

**Optimization of SBE-TAGS technology for the rapid and cost-effective genotyping of single nucleotide polymorphisms in ADHD.** *J.L. Stone, M.N. Ogdie, V. Kustanovich, L. Crawford, J. Kimura, S. Nelson.* Department of Human Genetics, UCLA, Los Angeles, CA.

In the sphere of human genetics where SNP markers have now become plentiful, the key issues in large-scale implementation of single nucleotide polymorphism (SNP) typing strategies revolves around the cost, accuracy, and speed of SNP genotyping. The genetic elucidation of complex traits is demanding the genotyping of large numbers of markers on hundreds of individuals per study both to follow up linkage studies and to identify association/linkage disequilibrium. In an effort to extend current genotyping methods, we have further optimized a method for high-throughput SNP genotyping, which is both cost effective and accurate.

The method is based on single nucleotide primer extension of tagged oligonucleotides (SBE-TAG). Each tagged SBE primer has a unique tag at its 5' end. Up to 100 such tagged SBE primers extension reactions can be multiplexed such that a single fluorescently labeled ddNTP is incorporated into each tagged SBE primer to score the allele. Which allele has been extended with which ddNTP is decoded by hybridization to a standard array of the 100 different reverse complements of the tags. In order to make this reaction simpler and more robust, we have created inexpensive spotted oligonucleotide arrays on standard glass slides that allow 16 different individuals in discrete wells to be genotyped per slide using standard multichannel pipettors. Second, we have introduced a two-dye system utilizing only cy3 and cy5, which simplifies the assay and make it readily compatible with existing microarray scanner technologies. We have developed software for the direct computation of SNP types from image data in a batch process mode.

As a stringent test of the system, we are applying this assay to genotyping candidate alleles and candidate regions in 250 multiplex families with attention deficit and hyperactivity disorder.

**Mapping genes for human mitochondrial ribosomal proteins.** *T.W. O'Brien*<sup>1</sup>, *H.-R. Graack*<sup>2</sup>, *N. Fischel-Ghodsian*<sup>3</sup>, *E.B. Mougey*<sup>4</sup>, *B.A. Maguire*<sup>4</sup>, *B. Wittmann-Liebold*<sup>5</sup>, *D.P. Nierlich*<sup>6</sup>, *J.E. Sylvester*<sup>4</sup>. 1) Biochemistry & Molec Biol, Univ Florida Hlth Sci Ctr, Gainesville, FL; 2) Max Planck Institute for Infection Biology, Berlin, Germany; 3) Cedars-Sinai Medical Center, Los Angeles, CA; 4) Nemour's Children's Clinic, Jacksonville, Fl; 5) Max-Delbruck-Center for Molecular Medicine, Berlin, Germany; 6) UCLA, Los Angeles, CA.

Mitochondrial DNA encodes tRNAs and rRNAs, but the other genes for the mitochondrial translation system are found in nuclear DNA. Upward of 85 mammalian mitochondrial ribosomal proteins (MRPs) are imported into mitochondria where they assemble into ribosomes that are responsible for translating the 13 mRNAs for essential proteins of the oxidative phosphorylation system. Since mutations in mitochondrial tRNA and rRNA can cause various pathological states, we hypothesize that mutations in MRP genes are also candidates for human disorders. Our approach is to use N-terminal and internal amino acid sequence data obtained from purified bovine MRPs (O'Brien, et al. (2000) J Biol Chem. 275: 18153) to search EST databases. Chromosome map positions for the MRP genes are ascertained by *in situ* hybridization (FISH) with genomic sequences and/or by using *in silico* methods to search Genbank and GeneMap through NCBI. At present, we have over 45 different human MRPs at various stages of characterization. We are currently investigating one MRP as a potential candidate for Russell-Silver Syndrome (RSS), a dwarfism characterized by low birth weight and lateral asymmetry, characteristics that are consistent with reduced mitochondrial function. DNA from RSS patients is being analyzed for possible mutations in MRPs. In addition to studying their clinical relevance, long term characterization of MRP genes should lead to important insights into mammalian evolution, coordinate regulation of nuclear and mitochondrial gene expression, and ribosome function. This work is being done as part of the Mammalian Mitochondrial Ribosomal Consortium and is supported by NIH/NIDCD grant RO1DC04092 and the Nemours Research Program.

**Misexpression of 50 uncharacterized *Drosophila* homologs to human disease genes reveals insight into gene function.** *D. Huffman, L.T. Reiter, E. Bier.* Biology, UCSD, La Jolla, CA.

We conducted a large scale BLAST analysis of human disease genes associated with known mutant alleles against the complete *Drosophila melanogaster* genome. This analysis, available as a searchable database called [Homophila](#), revealed that 77% of these genes have *Drosophila* counterparts. A goal of creating this database was to identify disease gene homologs in flies which might be studied effectively using powerful molecular genetic tools available in *Drosophila*. We were interested in determining the consequences of misexpression on a select group of 50 of these *Drosophila* genes, which have no known mutant alleles. These genes were selected due to their proximity to *p*-elements called an enhancer promoter elements (EP) which contains 14 copies of the yeast GAL4 upstream activating sequences (UAS). Using these stocks it is possible to misexpress the gene of interest by crossing the EP line to a stock expressing the GAL4 transactivator protein in one of many available patterns. Each of the 50 selected EP lines were crossed to a set of representative GAL4 driver stocks expressing GAL4 in various patterns during development: GMR drives expression in the eye; MS1096 and *dpp*<sup>disk</sup> in the wing; and *paired* drives expression in every other embryonic body segment. The progeny of these UASxGAL4 crosses were then scored for visible phenotypes or lethality that would be suitable for use in screens for identifying second site enhancer or suppressor mutations. Of the 50 EP lines screened, two showed moderate to severe phenotypes with one or more of the four GAL4 drivers. One example is the *Drosophila* homolog of the human disease gene ATR-X, which causes X-linked alpha-thalassemia. Overexpression of this gene in the wing causes mis-shaped wings with ectopic bristle formation while its overexpression in the eye results in a rough eye phenotype. The human counterpart of this gene is thought to be a member of the SNF2 subfamily of ATPase helicases. We anticipate that through misexpression and loss of function studies of human disease associated genes in *Drosophila* we will rapidly gain insight into disease gene function..

**High-Speed Detection of  $\alpha$ 1-Antitrypsin Deficiency Alleles PiZ, PiS and Adjacent Sequence Variations with Hybridization Probes and Real-Time PCR on the LightCycler.** C. Aslanidis, G. Schmitz. Inst Clinical Chem/Lab Med, Univ Regensburg, Regensburg, Germany.

$\alpha$ 1-Protease inhibitor I ( $\alpha$ 1-antitrypsin,AT) is the main serum inhibitor of proteolytic enzymes. In AT deficiency, enzymes like neutrophil elastase can damage the lung tissues leading to pulmonary emphysema. More than 90 different alleles have been identified so far for the  $\alpha$ 1-protease inhibitor I (PI) gene. The three most important variants are type M (90% of population), and type S (Pi\*S) and Z (Pi\*Z), two of the commoner abnormal variants. Homozygotes of type Z may develop pulmonary emphysema or hepatic cirrhosis. SZ-heterozygotes are less severely affected. The underlying mutations are a GAG to AAG (Glu342Lys) for Pi\*Z and a GAA to GTA (Glu264Val) for Pi\*S.

To date different phenotypes have been characterized by isoelectric focusing, or lately, by DNA-based technologies like PCR-RFLP. These technologies are time consuming and cumbersome. We have used PCR in glass capillaries in combination with hybridization probes capable of fluorescence resonance energy transfer (LightCycler) and melting curves for mutation detection in Pi\*Z and Pi\*S. Amplification and allele characterization is completed within 25-30 min. The melting peaks in both applications are 6 °C and 7 °C apart and allow unequivocal identification of the genotypes. In addition, using one hybridization probe for the PiZ allele (Glu342Lys), unambiguous identification of an adjacent polymorphism (one nucleotide apart) is facilitated. A C to G transversion leads to a Asp341Glu amino acid change. The resulting C-C mismatch in the hybridization probe leads to a T<sub>m</sub>-shift of 10 °C. The protocol used here does not involve the digestion of PCR-products with restriction enzymes followed by gel electrophoresis. PCR contamination concerns due to sample handling are eliminated.

**A high through-put mutation detection technique for identification of genes implicated in cancer.** *H.R. Molloy<sup>1</sup>, G.R. Bignell<sup>1</sup>, C.J. Cox<sup>1</sup>, P.J. Stephens<sup>1</sup>, M.J. Dronsfield<sup>2</sup>, P.A. Futreal<sup>1</sup>, M.R. Stratton<sup>1</sup>, R. Wooster<sup>1</sup>.* 1) The Cancer Genome Project, The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, England; 2) Applied Biosystems, Warrington, UK.

In the search for genes involved in the development of cancer we are using the human genome sequence to systematically screen all coding exons in the genome for somatic mutations. The current estimate of the number of genes in the genome is 33,000 and an average gene will require 12 STSs to cover all the coding exons. Therefore, to analyse the entire genome from one primary tumour, together with its corresponding normal DNA control, will involve screening around 800,000 PCR fragments. We have developed a high through-put mutation detection technique capable of detecting base substitutions and small insertions and deletions for this work. The technique termed Conformation Sensitive Capillary Electrophoresis (CSCE), is based around heteroduplex analysis of fluorescent labelled PCR fragments analysed by capillary electrophoresis on an ABI PRISM 3100 Genetic Analyser. Heteroduplex peaks are detected by a shift in the migration of the peak relative to homoduplexes. To assess the sensitivity of this technique we have screened 43 STSs containing known SNPs against a panel of 24 normal DNAs. The results were compared to direct sequencing of all the STSs. CSCE analysis detected all insertion/deletion polymorphisms and 83 out of the 88 base substitutions present in the fragments screened, giving a sensitivity of greater than 94%. Using this technique a number of different fragments can be analysed simultaneously by multiplexing on both size and fluorescent label. We estimate that using 25 3100 machines it will take 10 days to screen the entire genome of one tumour sample. In addition to cancer genetics, this method is also applicable to large-scale mutation screening for other diseases, both in situations where there is a well localised disease gene and where the disease alleles can only be mapped to large genomic intervals.



**SNP analysis from ultra low levels of human genomic DNA using a homogeneous Invader®/PCR assay.** A.A. Lukowiak, J.G. Hall, V.I. Lyamichev, B.P. Neri. Third Wave Technologies, Madison, WI.

The Invader assay allows for the direct scoring of single nucleotide polymorphisms (SNPs) using as little as 10's of nanograms of human genomic DNA without any target amplification. Although this limit of detection is sufficient for many applications, studies with limited genomic samples or which require large scale SNP scoring may demand pre-amplification of DNA prior to SNP analysis. Pre-amplification of genomic targets using PCR would not only provide improvements in detection, but would furnish a flexible format for the discrimination of pseudogenes and the identification of haplotypes during SNP detection as well. Here we describe a novel method for using PCR in combination with the Invader assay in a homogeneous format. This system provides significant improvements in sensitivity and is readily adaptable to high throughput applications using 96 and 384 well formats. In addition, we have investigated the possibility of using multiplex PCR in combination with the Invader assay for the pre-amplification of multiple targets from a single genomic sample.

**High-throughput mutation detection and screening using MegaBACE™ Capillary Array Instrument for Genetic Analysis.** *M. Minarik*<sup>1</sup>, *J. Bjørheim*<sup>2</sup>, *P.O. Ekstrøm*<sup>2</sup>, *K.M. Dains*<sup>1</sup>. 1) R&D, Molecular Dynamics, Sunnyvale, CA; 2) Dept. of Surgical Oncology, The Norwegian Radiumhospital, Oslo, Norway.

Over the past decade, DNA mutation detection and screening has become a valuable tool for genetic testing and clinical diagnostics. A number of point mutations within genes are being used as markers indicating the occurrence of diseases such as breast cancer, colorectal cancer, cardiovascular disease and others. Detection of low-level point mutations is a key factor for disease prevention through early diagnosis. The current methods of testing widely accepted in the clinical community are based on DNA sequencing and allele-specific PCR, but these methods are expensive and often lack sufficient sensitivity. Other techniques, such as denaturing HPLC (dHPLC), restriction fragment length polymorphism (RFLP) or single-stranded conformation polymorphism (SSCP) are currently low throughput assays, prohibiting large-scale prevention screening. Here, we demonstrate a high-throughput approach for mutation detection using a technique based on modified heteroduplex analysis. Using the MegaBACE, a capillary electrophoresis instrument for genetic analysis, we apply temporal temperature gradients to resolve heteroduplexes of multiple point mutations for up to 384 samples in a single run. We will present data from colorectal carcinoma patients screened for k-ras and p53 mutations using this new, high-throughput.

**Characterisation of Cytochrome p450 Polymorphisms Using the Invader System ®.** *M. Neville<sup>1</sup>, R. Walton<sup>2</sup>, B. Aizenstein<sup>1</sup>, M. Maguire<sup>1</sup>, R. Seltzer<sup>3</sup>, K. Hogan<sup>3</sup>, B. Neri<sup>1</sup>, M. de Arruda<sup>1</sup>.* 1) Third Wave Technologies, Inc, Madison, WI; 2) Oxford University, Oxford, UK; 3) University of Wisconsin, Madison, WI.

The cytochrome p450 (CYP) superfamily comprises a group of enzymes that play an essential role in the biotransformation of medically relevant compounds. Approximately 40% of CYP isoforms are polymorphic, including CYP1A2, 3A4, 2B6, 2D6, 2C9, AND 2C19. Accurate genotyping of patients for these and other p450 loci is of increasing interest because allelic variants may lead to loss of efficacy or toxic accumulation. These consequences are particularly pronounced in the perioperative interval with multiple low therapeutic ratio substrates competing for shared CYP pathways.

The Invader genetic analysis system developed by Third Wave Technologies, is a homogeneous, isothermal, FRET based assay. The technology employs a 3' invading oligonucleotide and a partially overlapping 5' primary probe oligonucleotide that form a specific ensemble when hybridised to a complementary DNA template. Upon recognition of this invasive structure, a Cleavase enzyme cleaves off the 5' flap from the primary probe oligonucleotide. In a simultaneous reaction, the cleaved flaps hybridise to a FRET cassette generating the substrate to the Cleavase Enzyme. Cleavage of the FRET cassette generates fluorescence, which is easily detected by standard plate readers. The Invader assay is highly specific and able to resolve single nucleotide polymorphisms with exquisite accuracy. ®

Here, we describe the analytical validity of a panel of CYP genetic assays comparing the Invader system to PCR-RFLP, and present allele frequency data from a panel of ethnically diverse individuals.

**High throughput multiplex genotyping using chimeric LNA (Locked Nucleic Acids)/DNA oligos immobilized on a polymer microchip.** *H. Vissing, A.T. Nielsen, M. Noerholm, P. Mouritzen, P.E. Hojby, S. Pedersen, Y. Choleva, M.S. Andersen, J.G. Kolberg, K.H. Haagesen, L. Kongsbak.* Bioinformatics/Genomics, Euray, EXIQON A/S, Vedbaek, Denmark.

A new approach for nucleic acid variation detection has been developed by taking advantage of three novel technologies: 1) LNA (Locked Nucleic Acids) 2) anthraquinones for photoactivated cross-linking and 3) a polymer microchip platform. LNA, a bicyclic DNA analogue, has proven very useful in hybridizations due to the exceptional high specificity and affinity. Short chimeras of LNA and DNA with the length of 12 nucleotides allow discrimination of single base mutations in microchip-based hybridizations. The anthraquinone phosphoramidites facilitate an efficient and robust polarised coupling of the capture probes or target to the polymer platform, which generates high quality hybridization spots with a diameter of less than 50 nm and a high-density homogeneous distribution. The use of chimeric LNA and DNA oligos as PCR primers facilitates specific multiplex PCR target amplification as well as allele specific amplification for haplotype assessment.

We have applied these technologies for two different designs of microchips. The first one is a direct multiplex SNP detection targeting more than 100 SNPs related to MODY (Mature Onset Diabetes Mellitus) and obesity. The second is a reverse format in which multiplex gene target amplicons are spotted on microchips and assayed for multiple SNPs. More than 10.000 patients can be genotyped on a single microchip and also allows for haplotype assessment. An example of this is SNP detection and haplotype assessment in Human Melanoma Inhibitory Activity like Gene (MIAL). In order to optimize the design of microchips, we have developed a software package integrating microchip design, database and neural network analysis.

**Single Nucleotide Polymorphism Typing Using Dual-Color AlphaScreen Technology.** *L. Beaudet<sup>1</sup>, J. Lian<sup>1</sup>, B. Breton<sup>1</sup>, M.-E. Caruso<sup>1</sup>, M.L. Budarf<sup>2</sup>.* 1) BioSignal Packard Inc., 1744 William #600 Montreal, QC, Canada, H3J 1R4; 2) Division of Human Genetics, Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

In AlphaScreen homogeneous assays, a highly amplified signal is generated when donor and acceptor beads are brought into proximity. The coupling of AlphaScreen to PCR product detection has allowed the development of a high-throughput SNP analysis platform (Beaudet et al., *Genome Research* 2001, **11** (4): 600). In SNP assays, AlphaScreen beads and probes are combined with the PCR reagents prior to amplification. The genotypes are analyzed after amplification without the need to purify the PCR products or to perform additional enzymatic reactions.

Recently, a second donor bead has been developed, which is activated at 780 nm, compared to 680 nm for the original donor bead. When this bead is used in conjunction with the original donor and acceptor beads, two different targets can be detected in a single well. For SNP analysis, the use of two different donor beads allows the detection of the two SNP alleles in the same well. This approach increases throughput, reduces significantly genotyping costs and, and increases the accuracy of genotyping.

We have developed several homogeneous genotyping assays using dual color AlphaScreen and results will be presented on the late onset Alzheimer marker APOE and on CYP3A polymorphisms that may be implicated in altered drug metabolism.

**High Throughput SNP scoring using the Invader® Operating System.** *M.W. Atilas, S. Law, R. Roeven, K. Lockwood, J. Koebel, R. Grygiel, B. Neri, M. de Arruda.* Third Wave Technologies, Madison, WI.

Sequencing the Human Genome has opened many opportunities to understand diseases and improve patient care. SNP discovery and disease association studies are important steps to achieve the ultimate goal of personalized medicine. To help accelerate this process, Third Wave Technologies, Inc. provides dried generic reagents necessary for SNP genotyping using the Invader® Operation system. This highly flexible system allows users to increase their genotyping throughput by direct detection of nucleic acids without the need of previous target amplification. The Invader assay is homogenous and isothermal, and is based on the specificity of the Cleavase® enzyme, which recognizes a specific structure formed when two allele specific oligonucleotides hybridize to a given target. Signal detection is based on Fluorescence Resonance Energy Transfer (FRET), and uses two reporter dyes with non-overlapping fluorescence spectra, which allows detection of both alleles in a single well using standard fluorescent plate readers. The simplicity of the assay permits the reactions to be set up either manually or in a fully automated system. The generic Invader reagents (Cleavase enzyme, detection cassettes, and buffer) are provided dried in microtiter plates (96- or 384-well). The assay entails a short set-up, which requires the addition of allele specific oligonucleotides,  $MgCl_2$  and pre-denatured DNA, a 4 H incubation at 63°C and an additional 10-15 minutes of data acquisition and analysis. The 96-well format requires 75-100 ng of DNA in a 15ml reaction, and the 384-well format requires as little as 20-40 ng of DNA in a 6ml reaction. The different formats provide users flexibility in terms of throughput and equipment required. Results obtained from fully automated assays will be shown using 96- and 384-well biplex assays.

Program Nr: 1689 from the 2001 ASHG Annual Meeting

**Simultaneous Genotyping of More Than 1000 SNPs with the SNPcode™ Platform.** *M.A. Donaldson, J.A. Lathrop, W.M. Ankener, J.F. Studebaker, S.V. Alfisi, M.S. Philips, C.A. Gelfand, M.T. Boyce-Jacino.* Assay Research, Orchid BioSciences, Inc., Princeton, NJ.

The task of elucidating the human genome sequence has led to a dauntingly wide range of projects that use the genome sequence as a starting point, such as genotyping of single nucleotide polymorphisms (SNPs). Many SNP studies, for example correlating genotype to medically relevant conditions, require detection systems enabling thousands of SNPs per sample to be genotyped in a low cost, robust fashion. The SNPcode™ system developed by Orchid BioSciences, Inc. couples SNP-IT™ genotyping technology with the Affymetrix GenFlex™ platform to create a versatile high-density SNP scoring system. The inherent generic design of the chip allows the user to adapt any SNP loci of interest to this platform. In the assay, multiplex PCR is followed by solution phase SNP-IT primer extension. The SNP-IT products are then hybridized to the GenFlex chip, the sorting mechanism for the multiplexed reactions. During SNPcode product development we demonstrated that greater than 98% of all validated assays result in a genotype call with greater than 99% reproducibility.

Currently Orchid is developing panels of SNPs using the SNPcode system. PCR and SNP-IT reactions are routinely tested in 12-plex with 96 reactions pooled together for analysis on a single chip. Each reaction tests 12 unique SNPs for a single sample thereby enabling analysis of over 1100 SNPs for a single sample in one assay. Primer sets are individually designed for each SNP and panels are assembled into functional families based on the needs of the individual project. Results of various 1100-plex assays will be presented. At mid-year, more than 12 panels have been tested resulting in nearly 10,000 functional assays. By the end of 2001, Orchid plans to have more than 50,000 SNPs validated across three ethnically diverse populations, as part of Orchid's collaboration with The SNP Consortium. Orchid expects to develop SNP panels for chromosome mapping, drug metabolism (ADMET), and disease correlation.

**SNP Identification, Validation, and Scoring with the Multi-Functional CEQ™ 2000XL DNA Analysis System.**

*H.H. Wai, R.M. Oda.* Bioresearch Sys. Dev. Ctr., Beckman Coulter, Inc., Fullerton, CA.

The study of genetic variations in individual genomes is critical to understanding the biological mechanisms that lie behind susceptibility and predisposition to many diseases. Finding markers that are closely linked to these genetic predispositions could enhance the success of diagnosis and treatment of diseases that have a significant genetic component. Single Nucleotide Polymorphisms (SNPs) are located throughout the genome. Depending on the level of detail required, SNPs can be finely mapped with regular interval on different chromosomes. These finely mapped SNPs can then be used as molecular markers to establish accurate linkage of polymorphisms to genes and possible diseases. It is this accurate linkage aspect that can catapult SNP analysis into an extremely powerful and fast tool for many systematic studies of genes, diseases, and etc. Beckman Coulter's CEQ 2000XL is a multi-functional DNA Analysis System that can be used in SNP identification, validation, and scoring. The CEQ 2000XL can sequence different individual or pooled samples to identify potential SNPs. Sequencing the same genetic region of additional related and unrelated individuals can validate or invalidate any potential SNP. Once the biological importance of a SNP has been established, the CEQ 2000XL can be also used to accurately and efficiently score the validated SNPs in different individuals.



**Detection of Single Nucleotide Polymorphisms by MALDI-TOF Mass Spectrometry.** *P.M. Vallone, J.M. Butler.*  
Biotechnology Division, Natl Inst Standards/Technology, Gaithersburg, MD.

Single nucleotide polymorphisms (SNPs) are the most frequent form of DNA sequence variation in the human genome and are becoming increasingly useful as genetic markers for genome mapping studies, medical diagnostics, and human identity testing. The primer extension reaction is a commonly employed molecular biology assay for genotyping a known single nucleotide polymorphism marker in genomic DNA. In a basic primer extension assay a short (less than 30 bases) DNA oligonucleotide or primer is extended by a single nucleotide unit. The identity of the extended base allows the sample to be accurately genotyped.

One technique for SNP detection currently employed relies on the mass resolution between a primer and its single base extension product(s) utilizing Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry or MALDI-TOF MS. The speed of data collection by this technique is on the order of 5 sec per sample and has the potential for high throughput when interfaced with a robotic system and automated data collection/analysis. Genotyping results obtained from the basic primer extension assay, the GOOD assay and primer extension using a chemically cleavable primer will be presented. Comparisons of the primer extension assays will be addressed as well as issues pertaining to high throughput MALDI-TOF MS data collection and interpretation. Genotyping examples will be illustrated using human identity genetic markers located on the Y chromosome and in the mitochondrial genome.

**Improved genotyping of SNPs by double-discrimination allele-specific real-time PCR.** *J. Zhang, J. Li, R.M. Myers.*  
Dept. of Genetics, Stanford University School of Medicine, Stanford, CA 94305-5120.

While the Human Genome Project has made the initial identification of single nucleotide polymorphisms, or "SNPs", very efficient, robust methods for genotyping in a large number of genomic DNA samples are still in a state of development. The requirements for a useful technique are a high degree of accuracy, reproducibility, ease of use, and a reasonable cost per genotype. One of the difficulties in genotyping is accurately distinguishing heterozygotes from both homozygotes, and special attention must be paid to each SNP assay to be sure these conditions are met when the technique is applied on a large scale. In this study, we have developed a variation on the allele-specific PCR (AS-PCR) method, a commonly used technique in which one of the PCR primers in a pair contains the variable base at its 3' end. Standard AS-PCR is rapid and inexpensive to use, but is often unreliable for heterozygote detection. In the improvement we developed, two distinct pairs of regular primers are used. In one pair, both forward and reverse primers immediately match one allele at their 3' terminal bases, such that the two 3' ends of the primers actually overlap by one base pair. The second pair is similar, but the two primers match the other allele at their 3' terminal bases. We combine this "double discrimination" set of oligonucleotids with the use of real-time PCR, monitoring the progress of the amplification reaction with the inexpensive intercalating dye, SYBR-Green. Genotypes were correctly determined in 25 different SNPs by differences in the threshold cycle numbers of the two alleles. Without requiring the expensive fluorescent probes or extensive handling, this method provides an accurate, inexpensive and high throughput method to genotype SNPs in the scale suitable for the lab or median size research project.

**Microarray transcriptional profiling of the adaptive response in human lymphoblastoid cells..** *M.A. Coleman<sup>1</sup>, E. Yin<sup>1</sup>, B.J. Marsh<sup>1</sup>, L.E. Peterson<sup>2</sup>, K. Sorensen<sup>1</sup>, J. Tucker<sup>1</sup>, A.J. Wyrobek<sup>1</sup>.* 1) Biology and Biotechnology Research Program. Lawrence Livermore National Laboratory, Livermore, CA; 2) Department of Medicine, Baylor College of Medicine, Houston, TX.

Exposure to a low dose of ionizing radiation is known to protect against a subsequent higher dose of radiation. This phenomenon, known as adaptive response (AR), has been attributed to DNA repair and stress response functions, yet the responsible pathways and genes are not well understood. Our project investigated variation in AR across lymphoblastoid cells from unrelated individuals (Corriel Repository) and compared their transcription profiles of cells using RNA sampled 4 hours after they were treated under non-adapting (2Gy exposure only) or adapting conditions (5cGy priming dose followed 6hrs later by 2Gy). AR was confirmed by the frequencies of first-division micronuclei. Significant adaptation was detected in 60% of experiments, synergism in 15%, and no effect in 25%. Using Affymetrix oligonucleotide arrays, ~2700 genes showed differences in transcription levels between adapting and nonadapting conditions. Of these, 211 genes showed consistent and significant responses across both cell lines, including 101 genes that were up-regulated and 110 that were down-regulated. Genes associated with adaptation include PKC and MAP kinase as well as several heat shock genes (HSP70 and DNAJ). Several genes involved in the adaptive response are also known as cell cycle control and DNA repair genes (e.g., ATM, RAD51C, PCNA, E2F1, p130 and G-cyclins), indicating that AR is linked to induced changes in these functions. Several of these changes have been confirmed by real-time PCR. In addition, numerous novel non-annotated genes were identified, warranting future investigations. These findings suggest that AR is not a reproducible genetic trait for individual cell lines, but when it occurs in cells from unrelated individuals, it appears to involve similar transcriptional changes across hundreds of common genes. [This work was conducted under the auspices of DOE by the University of California, LLNL under contract W-7405-ENG-48 with support from NIH (ES09117-02) and DOE (KP110202).].

**Factorial moments analysis of intermittency in gene expression on cDNA microarrays.** *L.E. Peterson*<sup>1</sup>, *K. Lau*<sup>2</sup>. 1) Dept. of Medicine, Baylor College of Medicine, Houston, TX; 2) Dept. of Physics, University of Houston, Houston, TX.

Intermittency occurs when quantities depend more on physical fluctuations than statistical fluctuations. The method of factorial moments has been used in high energy physics to search for intermittency. This paper describes use of factorial moments to search for intermittency in gene expression on cDNA microarrays which contain expression levels for thousands of genes under given experimental and disease conditions. Data used in this investigation included log expression ratios (*LERs*) for (a) 2,466 genes of the bacteria *S. Cerevisiae* on 79 microarrays representing different experiments and time periods [Eisen et al., PNAS. 95(25):14863-14868 (1998)] and (b) 9,706 genes on 60 microarrays representing 60 cancer cell lines [Ross et al., Nat. Genetics. 24:227-235 (2000)]. Results indicate that, as the number of bins increases in the frequency distribution of *LER*, gaps become visible within the tails and peaks. This suggests the possibility of intermittency. Calculation of scaled factorial moments, which remove Poissonian noise, also reflected varying amounts of intermittency on most of the arrays. This may suggest that certain environmental stressors or cancers result in clustering (self-similarity) of genes and their expression levels. That intermittency changed across the arrays may suggest the existence of long-range correlations whereby one or more regulating signals affected expression of a large number of genes. An implicit property of scaled factorial moments is that there is no contribution from frequency bins with less than two genes, since bins with a single gene cannot contain any correlates. In light of the above, our results may suggest the presence of one or more unique regulating signals causing self-similarity of genes whose expression are fractal-like. (L.E.P. supported by NCI grant CA-78199-04).

**L1 suppression display: a genomic approach to the isolation and characterization of young L1s.** *R.M. Badge, J.V. Moran.* Human Genetics, University of Michigan, Ann Arbor, MI.

The most active L1 retrotransposon subfamily in the human genome, Ta, comprises a small number of sequences (~550). A significant proportion (~25%) of Ta subset L1s are full length and ~50% can encode potentially functional polypeptides. Up to 90% of Ta insertions are polymorphic, so that genomic sequence databases contain only a fraction of extant diversity. In contrast older L1 sequences (pre-Ta and non-Ta) are extremely abundant (~850,000 copies). These sequences generally are fixed, 5' truncated, rearranged and mutated, and rarely encode open reading frames. To evaluate the impact of Ta L1s on genomic diversity, subfamily specific methods are required. Such a method, allele specific PCR (using Ta specific SNPs) requires sequence variation data, so a database of full-length L1 sequences was constructed. The non-redundant database comprises 242 full-length L1 sequences from the human genome working draft sequence, annotated for target site duplication, polyadenylation, transduction and coding potential status, as well as diagnostic sequence variants. While limited to high frequency and fixed insertions, the database provides an extensive collation of L1 sequence variation, and is informative with respect to phylogenetic and mechanistic relationships. Primers designed using this database allowed the specific isolation and characterization of Ta subfamily insertions by multiplex suppression PCR display. The method requires only small amounts of genomic DNA and generates individual specific displays of both 5' truncated and full-length Ta insertions. Several Ta insertions were characterised and their polymorphic status confirmed by locus specific PCR. The use of methylation sensitive restriction enzymes also enables this technique to probe genomic epigenetic variation associated with L1. In combination these approaches (database and display) have identified a novel non-Ta subfamily that carries a SNP associated with the most active (in cell culture) Ta sequences. This finding emphasizes the importance of studying active L1 retrotransposons in the context of human populations and the synergy possible between molecular biological and bioinformatic methods.

**Discovery and analysis of a new polymorphic human-specific family of LINE-1 retrotransposons.** *I. Ovchinnikov, G.D. Swergold.* Dept. Medicine, Columbia U., New York, NY.

Here we describe a new, polymorphic, human-specific LINE-1 subfamily that we call Tb. The youngest and most active autonomous retrotransposons in the human genome are members of a family called Ta (L1Hs-Ta). They are defined by the nucleotides ACA + G at diagnostic positions in the 3' UTR. L1s that amplified in the human genome during the primate radiation have GAG + A at these positions. Of the 14 known de novo human L1 insertions, one had nucleotides ACG + G. Elements with the ACG sequence have not been previously investigated in detail. We identified 117 ACG L1 elements in the Genbank NR database including 86 with the sequence ACG + G (previously called "pre-Ta") and 31 with the sequence ACG + A (herein named Tb). From this we calculate that the haploid human genome contains 311 pre-Ta and 98 Tb elements. A sequence alignment of Ta, pre-Ta, Tb, and primate (GAG) L1s revealed that Tb elements shared 7 of 27 diagnostic sites with GAG elements while pre-Ta elements shared only 2. Autosomal Tb elements had an average pairwise sequence divergence of 1.4% compared with 0.9% for pre-Ta elements. We calculated the time of maximal amplification of Tb elements to be 2.5 MYA compared with 2.0 MYA for pre-Ta elements. Only 14% of Tb elements were polymorphic in the present-day human population compared with 26% of pre-Ta elements. None of the Tb or pre-Ta insertions were found in chimpanzees, gorillas, or orangutans indicating that both of these subfamilies arose after the separation of humans and apes. Several other characteristics of the elements confirmed that Tb elements were older than pre-Ta elements. These include a shorter poly(A) tail, more frequent degradation of the polyadenylation signal, and more frequent existence of microsatellites in 3' flanking DNA. Phylogenetic analyses using neighbor joining, maximum parsimony, and maximum likelihood methods all yielded tree topologies that separated Tb and GAG elements into separate branches but grouped the Ta and pre-Ta elements together. In summary, Tb elements represent a newly defined subfamily of polymorphic retrotransposons that are, at present, the oldest known human-specific LINE-1 subfamily.

**Insights into the mechanism of human LINE-1 retrotransposition.** *G.D. Swergold<sup>1</sup>, I. Ovchinnikov<sup>1</sup>, H.H. Kazazian, Jr.<sup>2</sup>, E. Ostertag<sup>2</sup>.* 1) Dept. Medicine, Columbia Univ, New York, NY; 2) Dept. Genetics, University of Pennsylvania, Phil. PA.

The currently accepted model of human L1 (L1Hs) transposition is called Target Primed Reverse Transcription (TPRT) and is derived from detailed investigations of the R2Bm element in the silkworm. Evidence to support TPRT in L1Hs transposition is lacking. We analyzed the structure of a large number of L1Hs insertions in order to define important molecular details about L1Hs retrotransposition. Our findings are: 1) A polymorphic L1-Hs insertion bearing a 3' flanking DNA transduction was discovered by L1 display. Detailed analysis of the polymorphic transduction revealed that internal priming by the nicked target site was used for first-strand cDNA synthesis, and supports the use of TPRT in L1Hs transposition. 2) Analysis of 117 human-specific L1 insertions revealed that 26% were full-length, 43% had 5' terminal truncations, and 31% had both 5' terminal inversions and truncations. The length of the truncated elements were not random and favored short insertions. For insertions with inversions, both the 5' terminal inverted segments, and the 3' terminal non-inverted segments were also consistently short. These data suggest that the L1 reverse transcriptase is a non-processive enzyme. 3) Relatively few inversion events (11%) resulted in perfect ends without gaps or overlaps between the segments. Duplications were more common (31%) and were short (avg. 19 bp). Gaps were most common (58%), and while most were short, some were > 500 bp long. 4) For inversions, analysis of the target site sequences immediately 5' of the insertions revealed a statistically significant similarity to the minus strand of the L1 consensus sequence immediately downstream of the 5' terminal inverted segments. These data suggest that separate cDNA priming events are involved in the reverse transcription of the 3' terminal and 5' inverted segments. We present a modified model of TPRT that explains our results. The model predicts that L1Hs TPRT differs in important molecular details from R2Bm TPRT, and raises the possibility that L1Hs insertions may occur into locations of pre-existing double strand DNA breaks.

**Gene dosage-dependent effects in YAC transgenic mice carrying expanded alleles at the Machado-Joseph disease**

**locus.** *C.K. Cemal<sup>1</sup>, C. Carroll<sup>1</sup>, L. Lawrence<sup>2</sup>, M. Lowrie<sup>2</sup>, F. Scaravilli<sup>3</sup>, PK. Thomas<sup>3</sup>, C. Huxley<sup>1</sup>, S. Chamberlain<sup>1</sup>.*

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Machado-Joseph disease or spinocerebellar ataxia 3 (MJD/SCA3) is a progressive neurodegenerative disorder caused by pathological expansion of a CAG trinucleotide repeat motif located within exon 10 of the MJD1 gene. The generation of a representative mouse model for the disease should give a greater understanding of the role of repeat length, tissue specificity and level of expression on the associated pathology. We have generated a number of YAC transgenic lines differing with respect to transgene copy number and pathological allele size. The animals demonstrate a progressive cerebellar deficit, with reduced pelvic elevation accompanied by motor and sensory loss and hypotonia. Detailed phenotype analysis in the transgenic lines has resulted in the detection of a number of gene dosage-dependent abnormalities including reduced body weight, hypoactivity, reduced grip strength, incoordination and abnormal limb clasping reflexes. Disease severity is proportional to both the level of expression of the expanded protein and the size of the expanded repeat. In contrast, transgenic animals carrying the wild-type (CAG)<sub>15</sub> allele at the MJD1 locus appear completely normal at 24 months. The YAC transgene is transmitted to the predicted 50% of offspring and expressed in the appropriate manner. Further expansion of the CAG repeat is only detected on paternal transmission and contraction of the repeat is observed on maternal transmission, concurring with the human condition. The model therefore is representative of MJD with respect to pathology, gene dosage effects and repeat instability and should be a good system to test future therapeutic strategies for this disorder.



**Strategies for the identification of non-integer repeats in the automated Short Tandem Repeat (STR) genotyping.**

*M.D. Dobbs, H. Gull, D. Campbell.* Bioresearch Sys Dev Ctr, Beckman Coulter, Inc, Fullerton, CA.

A number of variables are known to contribute to the accuracy of sizing DNA fragments using gel electrophoresis, suggesting that absolute fragment lengths reported by different instrument systems, or reported on the same instrument system using different separation parameters may not be comparable (see ABRF poster at <http://162.129.76.21/Posterhtml/title.html>). Under constant conditions, it is therefore necessary to establish average observed sizes and their spacing for each genetic marker of interest. Using Beckman Coulters CEQ™ 2000XL DNA Analysis System, we genotyped 90 individuals from the Coriell Cell Repositories DNA Polymorphism Discovery Resource Collection (M90PDR) for the following nine human STR markers: D1S1679, D3S2387, D9S938, D9S2157, D11S1884, D14S599, D22S683, DXS7132 and GATA193A07. The markers were selected for previously documented high heterozygosity and the presence of non-integer repeats. Data sorting, filtering, and linear regression analysis were performed using Microsoft<sup>1</sup> Excel to model software enhancements for the current CEQ 2000XL. For complex allele patterns, automatically ignoring all but the tallest peaks in the traces facilitated the interpretation of both simple and complex allele patterns. Linear regression analysis was used to infer which alleles were true non-integer repeats versus fragments that belonged in integer repeat bins. The rules developed proved useful in reducing large fragment lists of STR genotype to data sets that represented primarily evenly spaced alleles, with smaller numbers of alleles that were in between repeats, but at unit nucleotide intervals. Based on the wide array of fragments observed at these STR loci, we developed a graphical user interface that provides a reliable way of identifying true alleles when they differ from the expected integer repeat size.

<sup>1</sup>All trademarks are property of their respective owners.

**A New Approach To Genotype Deconvolution Based On Experimental Studies Of PCR Slippage.** *D. Shinde*<sup>1</sup>, *Y. Lai*<sup>2</sup>, *F. Sun*<sup>2</sup>, *N. Arnheim*<sup>1</sup>. 1) Program in Molecular Biology; 2) Dept. of Mathematics, University of Southern California, Los Angeles, CA 90089.

Microsatellite DNA sequences are highly polymorphic and a useful source of genetic markers for genotyping, individual identification and detection of genomic instability. During microsatellite PCR, insertion and deletion mutations produce stutter products differing from the main PCR product by multiples of the repeat size often leading to problems in allele identification. To more fully understand the mutation process, we analyzed the PCR slippage products of dinucleotide repeats (dC.dA)<sub>n</sub>-(dG.dT)<sub>n</sub> and mononucleotide repeats (dA)<sub>n</sub>-(dT)<sub>n</sub> cloned in a pUC18 vector. Template sizes varied from 2-21 CA repeats and 4-16 A repeats. Data was generated on ~10 independent single molecules of each of the 19 different clone types using two rounds of nested PCR. The size and peak areas of the unchanged and stutter products were obtained by capillary electrophoresis. We built a mathematical model for PCR slippage assuming the mutation rate of a template at any cycle depends on the number of repeats. A maximum likelihood approach is used to estimate the mutation rate per repeat per PCR cycle, the expansion to contraction ratio and the threshold repeat number for stutter. The "per repeat" mutation rate for the CA tracts was  $1.9 \times 10^{-3}$ , and contractions were ten times more frequent than expansions. For A tracts the rate was  $1.26 \times 10^{-2}$  and contractions outnumbered expansions by 5 fold. The threshold for 'stutter' products was 5 repeats for poly-CA and 8 repeats for poly-A. In both cases, tracts containing less than or equal to 8-10 bp had undetectable per repeat mutation rates and may be related to the fact Taq DNA polymerase makes contacts with approximately the same number of nucleotides in the active site. This experimentally derived threshold value is consistent with estimates based on in silico analysis of microsatellite allele size distributions in the genomes of human and other organisms. Because the model was found to fit the data well, the ability of the model to deconvolute the stutter profiles generated from mixtures of alleles of similar sizes will be tested.

**Microarray expression analysis supports upregulation of genes on the single active X chromosome, as compared to autosomal genes.** *C.M. Disteche, T. Holzman, D.K. Nguyen, R. Bumgarner.* Depts Pathology, Microbiology and Molecular Biotechnology, Univ Washington, Seattle, WA.

S. Ohno predicted that "to escape a great peril" genes on the active X chromosome would have been upregulated during mammalian evolution to allow for X inactivation and to maintain balance of expression with the autosomes. We have previously shown that the *Clc4* gene located on the X chromosome in some species of mice and on an autosome in other species of mice was expressed from the active X locus at twice the expression level of the autosomal locus. To extend these findings to additional genes and to another species we have explored microarray expression data, which provide an overview of genome expression from each human chromosome. Studies done by others in yeast and in human have shown that an increase in copy number of a given gene or chromosome is generally accompanied by increased gene expression that can be detected by microarray analysis. Therefore, we reasoned that, if genes located on the X chromosome had indeed been upregulated to compensate for their hemizygous expression due to X inactivation, the average expression of X-linked genes versus autosomal genes would be equal on microarray expression data. Human cDNA microarrays were examined in terms of expression from the X chromosome clones versus the autosomes. Genes that were expressed at a low level were eliminated from the analysis. Our studies indicate that the level of expression from 36 genes located on the X chromosome does not differ on average from the expression of 1,028 genes located on autosomes. These findings support the notion of evolutionary upregulation of the single active X chromosome in mammals.

**Catechol-O-methyl transferase inhibitors in the treatment of inherited dopamine deficiency.** *A. Ponzone, S. Baglieri, G. Battistoni, L. Sorasio, A. Martini, S. Ferraris, M. Spada.* Pediatrics, University of Torino, Torino, Italy.

Most patients suffering from inborn errors of aromatic amino acid hydroxylation have to be treated with L-dopa, the hydroxylated precursor of dopamine. They include all the recessive and dominant forms of tetrahydrobiopterin deficiency and tyrosine hydroxylase deficiency. The limiting factor in this therapy depends on the short plasma half-life of the administered L-dopa and on the production of methylated catabolites after long treatments. Some benefit can be obtained by the administration of inhibitors of peripheral decarboxylases, sustained-release preparations, monoamine oxidase inhibitors. With this aim, we attempted the concurrent administration of entacapone, a reversible inhibitor of catechol-O-methyl transferase. Six patients suffering either from 6-pyruvoyl tetrahydropterin synthase or from dihydropteridine reductase deficiency were given entacapone, in addition to their standard therapy, at the dose of 15mg/Kg/day. The evaluation of the efficacy was based on the assessment of neuromotor performances and included the analysis of dopamine and prolactin blood profiles and of L-dopa kinetics. The use of entacapone resulted in a substantial improvement of neurotransmitter therapy, with prolonged L-dopa half-life and reduced methyl-dopa production, also allowing a 30% reduction of the daily dosage and number of administrations of L-dopa.

**Molecular analysis of two patients with 3-hydroxy-methyl-glutaric aciduria: identification of three novel mutations including one de novo mutation.** *J.Y. Wu<sup>1,2</sup>, C.F. Yang<sup>1,2</sup>, F.J. Tsai<sup>1,2,3</sup>*. 1) Dept Medical Research, China Medical Col Hosp, Taichung, Taiwan; 2) Dept Medical Gen, China Medical Col Hosp, Taichung, Taiwan; 3) Dept Pediatrics, China Medical Col Hosp, Taichung, Taiwan.

3-hydroxy-3-methylglutary-CoA lyase (HMG-CoA lyase, HL) deficiency gives rise to 3-hydroxy-3-methylglutaric aciduria. This disorder is one of the many inborn errors of CoA ester metabolism, and is of particular interest because the deficient enzyme (HL) plays a key role in ketone body, fatty acid and leucine metabolism. The disease is fatal in about 20% of cases. HL deficiency is transmitted as an autosomal recessive trait. In this study, two unrelated patients with HMG-CoA lyase deficiency are screened by direct sequencing of cDNA and genomic DNA. We identified three novel mutations in HL gene in these two patients. Patient 1 was characterized to have a deletion of exon 3 in direct sequencing of HL cDNA. Direct sequencing of genomic DNA indicated a G-to-A change at the first nucleotide in intron 3, which leads to aberrant splicing. The other mutation was not identified despite rigorous sequencing of all exons of HL gene. Patient 2 was found to carry a missense mutation in one allele and splicing mutation on the other allele. In segregation analysis, this patient was found to inherit the splicing mutation from the mother while the father did not carry the other identified missense mutation. Fraternity test indicated the father was indeed this patient's biological father. Several polymorphism markers around the HL locus were selected to confirm that this patient did inherit one allele from the mother and the other allele from the mother. This data indicated that this patient carry a de novo mutation in HL gene in the allele from the father.

**Parent mastery of heel/finger stick skills in PKU as a predictor of outcome.** *G.L. Arnold, J. DeLuca, E. Blakely.* Div Pediatric Genetics, Univ Rochester Sch Medicine, Rochester, NY.

Cognitive outcomes in children with Phenylketonuria (PKU) are strongly related to plasma phenylalanine (phe) concentrations, particularly in the first decade of life. Thus, outcome for affected children potentially might be improved by early identification of families at risk for the development of poor PKU management skills. We studied the ability of parents to master performance of the required weekly heel/finger stick for phe concentrations in the first year of life as a predictor of later phe control. We reviewed the charts of children with early and continuously treated classic PKU from the University of Rochester Inherited Metabolic Disorders Clinic. The group whose parents had achieved home heel/finger stick measurements in the first year of life were compared to those whose levels were performed by a medical professional instead of a parent.

There were 44 children who met study criteria. The group whose parents mastered the home draws was composed of 13 boys and 9 girls with mean age 8.2 yrs, and the group whose parents did not master this skill was composed of 17 boys and 5 girls with mean age 7.5 yrs. Median phe concentration from the prior year in the parent drawn group was 4.5 mg/dl and in the professional drawn group was 8.6 mg/dl ( $p < 0.05$ ). This relationship was even stronger for children under ten years old, where mean phe in the parent drawn group was 4.0 mg/dl, and was 6.9 mg/dl in the health professional drawn group ( $p < 0.002$ ). Further, six children from the professional drawn group required Child Protective Referrals for medical neglect, compared to none in the parent drawn group ( $p < 0.05$ ).

Parent mastery of heel/finger sticks for phe concentrations appears to be a strong predictor of outcome, particularly in the first decade. This is likely an indirect measure of family functioning including parental limit-setting, coping strategies, organization or other issues. Further studies are planned to determine which aspects of family functioning are impaired in families who fail to master this skill and if earlier intervention can improve outcome.

**Mutation analysis in families with X-linked metabolic disorders: adrenoleukodystrophy (X-ALD), ornithine carbamoyltransferase (OTC) deficiency and hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency.** *L. Dvorakova, E. Tietzeova, G. Storkanova, J. Zeman, E. Hrubá, E. Kostalova, I. Sebesta, S. Kmoch, M. Hrebíček.* Inst inherit Metabol Disorders, Prague 2, Czech Republic.

In families with gonosomal recessive disorders a reliable detection of carriers is important. To identify heterozygotes in affected families we have set up methods for mutation analysis in ABCD1 (gene for X-ALD), OTC and HPRT genes. As the mutations are usually private, we start the analysis by direct sequencing of PCR or RT/PCR products amplified from genomic DNA or cDNA of probands or obligatory heterozygotes. Subsequently, family members are genotyped by specific PCR-RFLP assays. X-ALD patients with all common phenotypes (5 ccALD, 4 AMN, 2 ADO, 3 asymptomatic) came from 12 unrelated families. We have found 12 mutations, 9 of them being novel. No genotype - phenotype correlation was found. We have analyzed mutations in 10 families with OTC deficiency. In 3 families with neonatal form two nonsense mutations and one missense mutation were found. In one OTC family the only patient is a symptomatic female heterozygous for a novel nonsense mutation. In 4 out of 6 families with late onset form of the disease we have identified a previously reported mutation, R277W. Up to now we have examined only one family with a partial HPRT deficiency. The proband is a symptomatic female. She is a heterozygote for previously described missense mutation V53A. Her father, who has symptoms of partial HPRT deficiency, is hemizygous for the mutation. In total we screened 65 family members from all affected families and identified 4 presymptomatic boys and 24 heterozygotes. In one X-ALD family mutation analysis served for prenatal diagnosis. Interestingly, we did not detect the mutation in 3 out of 18 examined mothers - obligatory heterozygotes. As the examined DNA is isolated from leukocytes, we cannot exclude the possibility of gonadal mosaicism, a phenomenon described in literature. Therefore, in the case of these mothers we strongly recommend genetic counseling and prenatal diagnosis in future pregnancies. (Supported by IGA MH grants NE5770-3-99 and NE 6557-3-01).

**Characterization of the human gene encoding Alpha-aminoadipate Aminotransferase (AAAT).** *D.L. Goh<sup>1</sup>, A. Patel<sup>2</sup>, G.H. Thomas<sup>2</sup>, M.T. Geraghty<sup>1</sup>.* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD.

In mammals, the degradation of L-lysine is a multi-step process that ends in the formation of acetyl-CoA. An intermediary step in this pathway involves the transamination of alpha-aminoadipate to alpha-ketoadipic acid. The rat gene encoding alpha-aminoadipate aminotransferase (KAT/AadAT) was previously identified. Using the BLAST algorithm, we identified a human EST contig homologous to the rat KAT/AadAT gene. The full length cDNA contains an open-reading frame of 1275 bp, predicted to encode 425 amino acids which contains a mitochondrial targeting signal as well as the expected pyridoxal-phosphate binding site. The human protein is 64% identical to the rat protein and 29% identical to its yeast counterpart. In Northern blot analysis, the cDNA hybridized to two transcripts, a major signal at ~2.9 kb and a minor signal at ~4.7 kb. Expression was highest in liver but was also seen in heart, brain, kidney, pancreas, prostate and testis. We further identified a human genomic BAC clone containing the AAAT gene, and using FISH confirmed the localization of this gene to chromosome 4q32.2. Using the BLAST alignment tool, we characterized the genomic structure of the gene, which consists of 12 exons spanning 40kb. Bacterial expression studies are currently underway to confirm the predicted function of this gene. Finally, while it has been suggested that patients with B6 responsive seizures might have a defect in this enzyme, we sequenced the cDNA of two patients and were unable to find any mutations in the AAAT gene.



**Human Cystathionine b-Synthase Gene is Coordinately Regulated with Proliferation by a Synergistic Interaction Between Sp1 and Sp3.** *K. Maclean, E. Kraus, J.P. Kraus.* Dept Pediatrics, C-233, UCHSC, Denver, CO, 80262.

Cystathionine b-synthase (CBS; L-serine hydro-lyase (adding homocysteine), EC 4.2.1.22) catalyzes the condensation of serine with homocysteine to form cystathionine, which is subsequently converted to cysteine. Homozygous CBS deficiency is the most common cause of classical homocystinuria, whilst partial deficiency of CBS has been proposed to cause mild hyperhomocysteinemia, considered an independent risk factor for arteriosclerosis. We have previously identified two separate promoters in the human CBS gene designated -1a and -1b, and have shown that they are coordinately regulated with proliferation. Deletion analysis and promoter assays in HepG2 cells using luciferase reporter constructs were used to define both the basal and minimal CBS -1b promoter regions. This promoter was found to be serum and fibroblast growth factor inducible and is down regulated by growth arrest and differentiation. Examination of CBS promoter activity in Sp1 negative *Drosophila* SL2 cells indicated that both CBS promoters have an obligate need for the redox sensitive transcription factor Sp1. Deletion analysis of the CBS -1b basal promoter indicated the presence of at least 3 key Sp1 sites that contribute to basal promoter activity. The CBS -1b minimal promoter contains one Sp1 binding site and when this site is mutagenized CBS promoter activity is abolished. Co-transfection of SL2 cells with the CBS -1b basal promoter with either Sp1 or Sp3 expression constructs leads to CBS promoter activity approximately 10-fold over background. However, co-transfection of the CBS -1b promoter with both Sp1 and Sp3 lead to a 70-fold increase of CBS activity indicating that regulation of the human CBS -1b promoter involves a positive synergistic interaction between these two transcription factors. When this transfection is repeated using the CBS-1b minimal promoter, the synergistic interaction is lost indicating that not all Sp1 sites in the CBS -1b promoter are functionally equivalent. Several key Sp1 sites in the -1b promoter have been identified and this regulation has been shown to be sensitive to intracellular redox as a function of cellular proliferation status.

**Identification of the gene encoding hydroxyproline oxidase and delineation of mutations responsible for hyperhydroxyprolinemia.** C. Hu<sup>1</sup>, W-W. Lin<sup>1</sup>, G. Steel<sup>1</sup>, H. Levy<sup>2</sup>, D. Valle<sup>1</sup>. 1) Inst Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Children's Hospital, Boston, MA.

Using the sequence of yeast and *Drosophila* proline oxidase to probe mammalian EST and genomic databases, we identified two homologous human genes: the first, *POX*, located at 22q11.2, encodes proline oxidase; the second, located at 19q13.1, by elimination, became a candidate for the gene (*HPOX*) encoding hydroxyproline oxidase. The protein products of these two genes have 33% amino acid identity. Hydroxyproline oxidase (HPOX) is a mitochondrial enzyme that catalyzes the first step in hydroxyproline degradation. Deficiency of HPOX is presumed on the basis of metabolite studies to be the primary defect in hyperhydroxyprolinemia (HHP), an autosomal recessive biochemical disorder characterized by a 10-fold accumulation of plasma hydroxyproline. The putative *HPOX* cDNA has a 1608 bp ORF encoding a protein of 536 residues with a predicted molecular weight of 58 kDa. Northern analysis showed expression in liver, kidney and heart. The *HPOX* gene has 12 exons distributed over 17 kb of genomic DNA. To confirm identity of the putative *HPOX* cDNA, we expressed it in Chinese hamster ovary cells and showed that it conferred hydroxyproline oxidase activity. To determine if HPOX mutations cause HHP, we surveyed the gene in a previously reported patient with HHP (Kim et al J Pediatr 130: 437, 1997). The proband, a 16 year old girl with a plasma hydroxyproline of 360mM (normal 0-50) and normal proline levels, is clinically asymptomatic. We amplified all 12 HPOX exons and their flanking splice sites from the proband and found that she is a compound heterozygote for a 1 bp deletion allele [H289fs(-1)] inherited from her father and an allele with an altered splice site in intron 6 (In6dnrG-1T) inherited from her mother. Both alleles are predicted to result in the loss of the C-terminal half of the protein. Our results confirm the identification of the human gene encoding hydroxyproline oxidase and show that mutations in this gene cause hyperhydroxyprolinemia.

**Mutational spectrum in the genes SLC3A1 and SLC7A9 in early manifesting cystinuria.** *C. Schmidt<sup>1</sup>, E. Botzenhart<sup>1</sup>, U. Vester<sup>2</sup>, P. Hoyer<sup>2</sup>, K. Zerres<sup>1</sup>, T. Eggermann<sup>1</sup>.* 1) RWTH Aachen, Institute of Human Genetics, Aachen, Germany; 2) Department for Pediatric Nephrology, Essen.

Cystinuria (OMIM 220100) is an inherited hyperaminoaciduria. The defective cystine transport across the renal brush border membrane leads to recurrent kidney stone formation. While mutations in the SLC3A1 gene cause type I cystinuria, patients with non-type I cystinuria harbour mutations in the SLC7A9 gene. Both gene products form a renal amino acid transporter. The different types can be distinguished biochemically by urinary amino acid profiles. Until now it has been assumed that type I patients show a more severe phenotype with earlier manifestation than non-type I patients. Goodyer et al. (*Kidney International*, Vol. 54, 56-61, 1998) demonstrated that patients with mutations in SLC3A1 form stones in their first decade of life while non type I patients only show increased urinary cystine values at this age. In this study we collected blood, plasma and urine samples of 22 cystinuria patients from 17 families. Patients' age ranged from nine months to 21 years, they and their relatives were classified biochemically by ion exchange chromatography. Genomic DNA was used for molecular genetic analyses (SSCA, restriction assays, sequencing) of both cystinuria genes. Our results indicate that the mutational spectrum in younger patients is similar to that in a previously studied group of unclassified patients with an older age of onset: 2/3 of the patients had mutations in SLC3A1, 1/3 in SLC7A9. In both groups M467T is the most frequent mutation. The study population also includes a sibship showing homozygosity for the same SLC3A1 mutation but with ages of onset of nine and 22 years. We conclude that stone formation depends on further modifying factors like urinary citrate or calcium concentration.

**Argininemia: A prospective study and two new cases.** *P.L. Plotner, H. Northrup.* Division of Medical Genetics, Department of Pediatrics, The University of Texas Medical School at Houston, Houston, TX.

Defects in any of the five urea cycle enzymes lead to disease and are collectively known as urea cycle defects. Urea cycle defects occur in about 1 in 30,000 live births. Argininemia is an autosomal recessive disease caused by a deficiency of the enzyme arginase, the last enzyme in the urea cycle. A deficiency of arginase leads to a build up of arginine and its metabolic by-products. Argininemia is the least frequently diagnosed urea cycle defect and its population incidence and prevalence are unknown. The most common clinical features of argininemia are neurologic: motor control and intellectual functioning. The presentation of motor deficits ranges from ataxia to spastic tetraplegia with spastic diplegia being, by far, the most common presentation (>90%).

To date 36 cases, representing 30 families, have been reported in the medical literature with Hispanic patients representing the largest ethnic group (46%). We report 2 unrelated patients, also of Hispanic ethnicity, that were diagnosed in our cerebral palsy clinic within the same year.

Identifying these two patients prompted a prospective search for others with argininemia within the same cerebral palsy clinic population. Including our two patients, 661 charts were reviewed with 136 (20%) included in the study. A plasma arginine level was obtained on 86 (63%) of the 136 study participants. No plasma arginine levels were diagnostic for argininemia except for those described above (1%). Based on this information, and a reported U.S. prevalence of cerebral palsy of 2/1000, we hypothesize that the population prevalence of argininemia is less than 1 per 350,000.

**PET scans abnormalities in adults with early-treated PKU.** *M.P. Wasserstein, S.E. Snyderman, C. Sansaricq, M.S. Buchsbaum.* Mount Sinai School of Medicine, NY, NY.

Classical phenylketonuria (PKU) may cause subtle neurocognitive defects despite early, adequate treatment. Understanding which regions of the brain are adversely affected may provide insight about the pathophysiology of these abnormalities. We used positron emission tomography (PET) with  $^{18}\text{F}$ -deoxyglucose in 12 adults (4 women, 8 men; ages 23 to 36) with early-treated PKU to evaluate if abnormalities exist in cerebral metabolic rate, if the abnormalities are related to concurrent plasma phenylalanine (Phe) levels, and if relationships exist between age-specific Phe levels and particular regions of abnormality. SPGR MRI with 1.2 mm slice thickness were reoriented to standard atlas position and PET coregistered. Relative metabolic rate at the center of positions identified in the atlas were assessed in all subjects. Repeated measures MANOVA was used to assess regional metabolic differences. Lifelong, age-specific phe levels were available for 9 patients. 10 patients had day-of-scan Phe levels  $>17$  mg/dl. Patients with PKU in comparison to normal, age- and sex-matched controls had lower relative metabolic rates in the executive area of the prefrontal cortex, but higher relative metabolic rates in the lateral temporal lobe and hippocampus (group by region interaction,  $F=8.46$ ,  $df=1,31$ ,  $p=0.006$ ). Patients had higher relative metabolic rates than normals in the putamen and the thalamus, and lower rates in the right caudate (group by structure by hemisphere interaction,  $F=6.05$ ,  $df=2, 62$ ,  $p=0.004$ ). Day-of-scan Phe correlated only with abnormalities in the globus pallidus (lower in patients than normals,  $r=0.75$ ,  $p<0.05$ ). An association was found between lower relative metabolic rates in Brodman's area 45, a late developing area involved in higher cognitive function, and Phe level during adolescence ( $r=-.78$ ,  $p<0.05$ ). These regional abnormalities correlate with some of the clinical features of early treated PKU, such as abnormal executive function (prefrontal cortex), and mood swings(temporal lobe). The finding of abnormalities in Brodman's area 45 suggest that the brain remains sensitive to the effects of phe longer than previously thought.

**Maple Syrup Urine Disease: Identification of a Novel Ashkenazi Jewish Mutation.** *S.E. Snyderman, M.P. Wasserstein, C. Sansaricq, R. Kornreich, G.A. Diaz.* Human Genetics, Mount Sinai School of Medicine, New York, NY.

Maple Syrup Urine Disease (MSUD) is a rare, autosomal recessive disorder of branched chain amino acid metabolism. The clinical course in classic MSUD is characterized by episodic metabolic decompensations that can result in severe neurologic damage or death. The disease is genetically heterogeneous and is caused by deficient activity of the mitochondrial multienzyme complex, branched chain A-keto acid dehydrogenase (BCKAD). Mutations in the E1 (composed of two E1A and two E1B subunits) or E2 components of BCKAD result in classic, intermediate, or intermittent MSUD. Whereas the frequency of MSUD in the general population is estimated to be approximately 1 in 185,000, its frequency in the Mennonite population is 1 in 176 due to a founder effect. We noted that a large percentage of MSUD families (10 of 34) followed in our clinic are of Ashkenazi Jewish descent, leading us to search for a common mutation within this group. A haplotype flanking the E1B subunit was found in homozygosity in two of five Ashkenazi Jewish patients screened, and in heterozygosity in three additional individuals. Additionally, 9 of 10 MSUD chromosomes studied shared a common allele at marker D6S251. Sequencing of the gene in a patient homozygous for the conserved haplotype revealed a novel homozygous 538G->C missense mutation in exon 5 predicted to result in a non-conservative substitution, R183P. Screening of the remaining patients for this mutation revealed that all chromosomes with the common allele at D6S251 carried the R183P mutation. This arginine residue has been completely conserved in the homologous proteins from bacteria through mammals and is located in a B strand that forms part of a K<sup>+</sup> ion-binding pocket close to the interface of the E1A and E1B subunits. The structural changes introduced by the mutation may thus interfere with the proper formation of the ion-binding pocket and/or with subunit interaction. In summary, we have identified a novel missense founder mutation in the E1B subunit causing MSUD in the Ashkenazi Jewish population. These findings enable the determination of allele frequency in this group and may facilitate carrier screening.

**Carnitine Metabolic Defects in Saudi Arabia.** *A.I. Al-Aqeel<sup>1</sup>, M.S. Rashed<sup>2</sup>, J.P.N. Ruiten<sup>3</sup>, L. Ijst<sup>3</sup>, R.J.A. Wanders<sup>3</sup>.*  
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Some 13 different inherited defects of mitochondrial fatty acid -oxidation have been described. Four of these affect the carnitine shuttle for the transport of long-chain fatty acids into the mitochondria, either intracellular uptake of carnitine of the enzymes carnitine palmitoyltransferase (CPT) I and II or carnitine acylcarnitine translocase (CACT). We report 5 affected Saudi patients, a product of first cousin marriage, with CPT I deficiency who presented with typical Reye-like syndrome with unconsciousness, hepatomegaly, hypoglycemia, hyperammonemia, very high liver enzymes and typical acylcarnitine profile. In one of these patients the disease was fulminant with hemophagocytic syndrome. With CACT deficiency we also report a five-month-old patient who presented on the second day of life with nystagmus and hyperammonemia and typical acylcarnitine profile. These patients had a unique molecular defect with a homozygous 1950 G-A transition in the cDNA encoding CPT1A, (G650D) and 713 A-C transversion (Q238R) in the CACT deficiency patient, which is conserved in other organisms. Expression studies in yeast has to be done to investigate the consequences of these mutations for the function of the enzymes. In conclusion, carnitine metabolic defects are treatable disorders with unique molecular defects in our Saudi patients.

**Impaired inducibility of superoxide dismutases in Friedreich's ataxia.** *V. Geromel<sup>1</sup>, K. Chantrel-Groussard<sup>1</sup>, H. Puccio<sup>2</sup>, M. Koenig<sup>2</sup>, A. Munnich<sup>1</sup>, A. Rotig<sup>1</sup>, P. Rustin<sup>1</sup>.* 1) INSERM U393, Hosp Necker, Paris, Paris, France; 2) Institut de Genetique et de Biologie Moleculaire et Cellulaire, INSERM, CNRS, 1 rue Laurent Fries, BP 163, 67404 Illkirch, France.

Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disease causing limb and gait ataxia and cardiomyopathy. The disease gene encodes a protein of unknown function, frataxin. In the vast majority of the patients, the loss of frataxin is due to a large GAA trinucleotide expansion in the first intron of the gene, which hampers the gene transcription by causing the formation of a sticky DNA structure. Decreased frataxin content results in a generalized deficiency of mitochondrial iron-sulfur protein activity that has been previously ascribed to mitochondrial iron overload. However, iron overload appears as a late event in the disease and in a mouse model as well. Here we show that neither superoxide dismutases nor the import iron machinery were induced by an endogenous oxidative stress in FRDA patient's fibroblasts as compared to control cells. Superoxide dismutase activity was not induced in heart of conditional frataxin-KO mice either. This suggests that continuous oxidative damages to iron-sulfur clusters, resulting from a impaired superoxide dismutase signaling, are causative of the mitochondrial deficiency and the long term mitochondrial iron overload occurring in FRDA.



**Deletion pattern of the STS gene and flanking sequences in Mexican patients with XLI.** *S.A. Cuevas<sup>1</sup>, A.L. Jimenez<sup>1</sup>, M. Valdes<sup>2</sup>, M.R. Rivera<sup>1</sup>, S.H. Kofman<sup>1</sup>.* 1) Genetica, Hospital General de Mexico, Mexico, D.F., Mexico; 2) Centro Nacional de Rehabilitacion (INO)Mexico, Mexico, D.F.

X-linked ichthyosis (XLI) is an inherited disorder due to steroid sulfatase deficiency. Most XLI patients (>90%) have a complete deletion of the STS gene and flanking sequences. The presence of low copy number repeats (G1.3 and CRI-S232) on either side of the STS gene, promoting unequal crossing over, seems to play a role in the high frequency of these interstitial deletions. In the present study, we analyzed 80 Mexican patients with XLI and complete deletion of the STS gene. STS activity was measured in the leukocytes using 7-[3H]-dehydroepiandrosterone sulfate as a substrate. Amplification of the regions telomeric-DXS89, DXS1139, DXS1130, 5'STS, 3'STS, DXS1133, DXS237, DXS1132, DXF22S1, DXS278-centromeric was performed through PCR. No STS activity was detected in the XLI patients (0.00 pmoles/mg protein/h). We observed 3 different deletion patterns. 57 patients showed a deletion at the sequence DXS1139 (5'end of the STS gene) that corresponded to the probe CRI-S232A2. 32 out of these patients presented the 3 rupture site at the sequence DXF22S1 (probe G1.3) and the rest 25 patients had the 3 breakpoint at the sequence DXS278 (probe CRI-S232B2). 23 patients had the breakpoints at several regions on either side of the STS gene as follows: 5'-3' ends, 2 patients; 5'end-DXS1131, 3 patients; 5'end-DXS1133, 1 patient; 5'end-DXS237, 1 patient; 5'end-DXF22S1, 1 patient; DXS1130-DXS1131, 2 patients; DXS1130-DXS237, 1 patient; DXS1130-DXF1132, 1 patient; DXS1130-DXF22S1, 3 patients; DXS1130-DXS278, 1 patient; DXS1139-DXS1131, 4 patients; DXS1139-DXS1133, 1 patient; DXS1139-DXS237, 2 patients. Although there was an important participation of low copy number repeats in the etiology of the 70% of our patients, we also had a high percent (30%) in which this low copy number repeats were not involved. These data indicate that more complex mechanisms, apart from homologous recombination, are participating in the ruptures sites of the STS gene in XLI patients in Mexican population.

**Chronic PDGF signaling in glioma cells modulates expression of multiple genes encoding receptors, signal transduction components, and pro-angiogenic factors, and may promote reciprocal paracrine communication between glioma cells and endothelia.** *M.A. Dressman<sup>1</sup>, E.C. Holland<sup>2</sup>, O. Grenet<sup>3</sup>, S.D. Chibout<sup>3</sup>, C. Lavedan<sup>1</sup>, M.H. Polymeropoulos<sup>1</sup>.* 1) Pharmacogenetics, Novartis Pharmaceuticals Corporation, Gaithersburg, MD; 2) Neurosurgery, Neurology, and Cell Biology, Memorial Sloan Kettering Cancer Center, New York, NY; 3) Toxicology, Novartis Pharmaceuticals Corporation, Basel, Switzerland.

Chronic autocrine loop stimulation by platelet derived growth factor (PDGF) has been proposed as a major contributor to glioma cell proliferation and induction of angiogenesis. Therefore, PDGF induced gene expression in glioma cells may provide insight into the molecular mechanisms of gliomagenesis. We have used expression profiling to identify genes that are regulated by PDGF signaling in the glioma cell line U373. Similarly, we generated expression profiles of VEGF signaling in cultured endothelial cells. A PDGFR specific regulated expression profile of 112 genes was identified providing a global snapshot of a dynamic process including successive stages of the tyrosine kinase signaling pathway, angiogenic genes and numerous receptor genes most likely involved in paracrine stimulation. The tyrosine kinase inhibitor PTK787 reversed the profile of 111 of these genes. Studying expression profiles of PDGFR signaling in parallel with VEGFR profiles suggested that chronic PDGFR signaling induces paracrine loops of glioma proliferation and induction of new blood vessel formation. Key members of this expression profile reside at the nodes of numerous signaling pathways and should be considered as therapeutic targets for diseases including cancer, stroke, hyperplasias, pulmonary fibrosis, psoriasis, and disorders of angiogenesis and wound healing.

**Tetrahydrobiopterin deficiency due to defective 6-pyruvoyl-tetrahydropterin synthase: negative charges at amino acid position 19 modulate enzyme activity.** *W. Leimbacher, N. Blau, B. Thony.* Dept Ped, Div Clin Chem/Biochem, Univ Zurich, Zurich, Switzerland.

We previously identified in a 6-pyruvoyltetrahydropterin synthase (PTPS)-deficient patient an inactive PTPS allele with an Arg16 to Cys codon mutation. Arg16 is located in the protein surface exposed phosphorylation motif Arg16-Arg-Ile-Ser-Phe, with Ser19 as the identified phosphorylation site for the cGMP protein kinase II, a serine/threonine protein kinase. Purification of recombinant PTPS-S19A from bacterial cells yielded an active enzyme that was kinetically (kcat/kM) indistinguishable from the wild-type enzyme. Adversely, upon transient transfection into COS-1 cells, the PTPS-S19A allele was stably expressed but not phosphorylated and had a reduced activity of 33% in comparison to wild-type PTPS (1). Since in vivo modification appears to be essential for normal activity, we investigated the function of phosphoserine19 of human PTPS in more detail by substitution of the Ser19 not only by alanine but also by an acidic residue. Replacement of Ser19 by aspartic acid mimics the kinase-modified PTPS-phosphoserine by replacing two negative charges from the phosphate group by one negatively charged side chain from Asp. Transient transfection into COS-1 cells of the PTPS mutant resulted not only in rescuing wild-type activation to 100%, but in hyperactivation of the Asp19 mutant to 146+18%. This demonstrates that alanine or acidic residues at position 19 in human PTPS modulate enzyme activity. (1) Scherer-Oppliger T, Leimbacher W, Blau N, and Thony B: Serine 19 of human 6-pyruvoyltetrahydropterin synthase is phosphorylated by cGMP protein kinase II, *J Biol Chem* 1999; 274:31341-31348.

**Gene expression profiling of renal cortex from STZ-induced diabetic rats.** *L.A. McLean<sup>1</sup>, D.L. Feldman<sup>2</sup>, L.M. Barnes<sup>1</sup>, T.C. Mogelesky<sup>2</sup>, W. Chen<sup>2</sup>, M.A. Dressman<sup>1</sup>.* 1) Pharmacogenetics, Novartis, Gaithersburg, MD; 2) MCD, Novartis, Summit, NJ.

The purpose of this study was to evaluate the molecular mechanisms associated with diabetic nephropathy by utilizing oligonucleotide microarray technology. RNA was extracted from renal cortex of three rats two weeks after treatment with streptozotocin (STZ) (a model of early diabetes) along with five untreated control animals. Subsequently synthesized and labeled cRNAs were hybridized to Affymetrix microarrays that represent over 8,000 full length genes and expressed sequence tags (ESTs). Several genes that have previously been demonstrated to be upregulated in diabetes were confirmed to have significantly increased gene expression levels in this study, including: mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase, gene 33 (mitogen-inducible gene-6), glucose transporter GLUT2, insulin-like growth factor binding protein 1 (IGFBP-1), and the diabetes-inducible cytochrome p450RLM6. Tamm-Horsfall protein, which is typically decreased in patients with diabetic nephropathy, was shown to have significantly lower expression levels in the STZ-treated rats, as were several other genes not previously associated with diabetes. Altered gene expression levels were found for several metabolic enzymes such as 2-hydroxyphytanoyl-CoA lyase; betaine homocysteine methyltransferase (BHMT); lipoprotein lipase; acetyl-CoA acyltransferase, 3-oxo acyl-CoA thiolase A; malic enzyme 1; and glutathione synthetase. Expression of several solute transport proteins were also differentially expressed in the diabetic animal model, including: kidney band 3 anion exchange protein, taurine/beta-alanine transporter, organic cation transporter 2 (OCT2), organic anion transporter (OATP), and the anion exchange protein 2 (AE2). Examination of expression levels of extracellular matrix (ECM)-associated genes revealed a significant ( $p < 0.05$ ) increase in laminin chain beta-2, the proteoglycan decorin, and the serine protease inhibitor leuserpin-2 in the STZ-treated animals. Enhanced gene expression of these ECM-related proteins may correlate to the hallmark ECM deposition seen later in STZ-induced diabetic nephropathy.

**Sub-cellular localization of tetrahydrobiopterin biosynthetic enzymes: nuclear localization of GTPCH and PTPS.** *B. Thony*<sup>1</sup>, *S. Laufs*<sup>2</sup>, *A. Resibois*<sup>3</sup>, *W. Leimbacher*<sup>1</sup>, *N. Blau*<sup>1</sup>. 1) Dept Ped, Div Clin Chem/Biochem, Univ Zurich, Zurich, Switzerland; 2) DKFZ, Im Neuheimer Feld, D-69120 Heidelberg, Germany; 3) Biological Chemistry Laboratory, Faculte de Medicine, Universite Libre de Bruxelles, Belgium.

Tetrahydrobiopterin cofactor deficiency comprises a heterogeneous group of disorders caused by mutations at one of the genes encoding enzymes involved in the biosynthesis or regeneration. The biosynthesis of tetrahydrobiopterin requires the enzymes GTP cyclohydrolase I (GTPCH), 6-pyruvoyl tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR). Upon studying the distribution of GTPCH and PTPS with polyclonal immune sera in cross-sections of rat brain and peripheral organs, strong nuclear staining was observed in neurons and cells of the kidney convoluted tubules. Cytoplasmic and nuclear staining at least for PTPS was also observed in transiently transfected COS-1 cells that do not have this enzyme (and GTPCH) endogenously present. To study the nature of this nuclear localization in more detail, we generated plasmids expressing recombinant Flag-tagged fusion proteins for the three human biosynthetic enzymes Flag-GTPCH, Flag-PTPS, and Flag-SR. Flag-peptide (DYKDDDDK) epitope-tagged enzymes can be specifically detected by a commercially available monoclonal antibody. Transiently transfected COS-1 cells expressing individual Flag-fusion proteins were fractionated into cytoplasmic and nuclear extracts, and analyzed for enzyme activity and cross reactivity by Western blot. All three enzymes were found in the cytoplasmic fraction, whereas only the Flag-GTPCH and Flag-PTPS were also located in the nucleus. Unexpectedly, enzymatic activity in the nuclear fraction was observed for Flag-GTPCH but not for Flag-PTPS. Furthermore, since no canonical nuclear localization signal is present in PTPS (and also in GTPCH), a series of amino acid alterations, and C- and N-terminal deletions of Flag-fusion proteins were tested to identify residues that may target the PTPS protein to the nucleus. Preliminary results suggest that correct folding and/or stable expression of PTPS is essential for nuclear localization.

**A novel heteroplasmic mtDNA mutation in a family with heterogeneous clinical presentations.** *M. Zeviani<sup>1</sup>, F. Carrara<sup>1</sup>, P. Corona<sup>1</sup>, E. Lamantea<sup>1</sup>, M. Greco<sup>1</sup>, M. Mora<sup>2</sup>, C. Mariotti<sup>3</sup>*. 1) Molecular Neurogenetics, Istituto Nazionale Neurologico, Milano, Italy; 2) Neuromuscular Disorders, Istituto Nazionale Neurologico, Milano, Italy; 3) Biochemistry & Genetics, Istituto Nazionale Neurologico, Milano, Italy.

The proband, a 36 year-old man, showed isolated, severe spastic paraparesis. One of his brothers had died at age 24 years of heart failure due to hypertrophic-dilative cardiomyopathy. Another brother, now 34 years old, is presently healthy. The proband's mother, aged 64 years, showed truncal ataxia, spastic paraparesis, slurred speech, severe hearing loss, and mental regression. A muscle biopsy performed in the proband failed to show the morphological abnormalities typical of mitochondrial disorders and the activities of respiratory chain complexes were normal. However, complex I and IV activities were 38% and 50% of controls' mean in the muscle homogenate of the mother, and 44% and 4% in that of the affected brother deceased of cardiac failure. In this sample, reduction to 54% of controls' mean was also found for complex V (mitochondrial ATPase). Sequence analysis of mtDNA from muscle tissue of the proband revealed a heteroplasmic mutation of the tRNA<sup>ile</sup> gene (G4284A). Allele-specific RFLP analysis and densitometry showed 55%, 80%, and 95% mutant heteroplasmy in the muscle mtDNA of the proband, his mother and affected brother, respectively. A lesser but still clearly detectable mutation load was present in other tissues of the affected members of the family, including urinary and oral mucosa, hair follicles, and blood. Mutation was absent in mtDNA from several tissues of the healthy brother, as well as in 100 lymphocyte mtDNA samples from Italian controls. Ten 143B.206 cybrid clones containing 100% mutant mtDNA were compared with ten cybrid clones containing 100% wt mtDNA, both derived from the proband. A significant reduction of complex IV activity normalized to that of citrate synthase was demonstrated in the mutant cybrids ( $20 \pm 7$ ) compared to the wt cybrids ( $33.8 \pm 7.6$ , Student's *t* test  $p < 0.0005$ ). These results indicate that the G4284A change in the tRNA<sup>ile</sup> gene is a novel pathogenic mutation of mtDNA.

**The Pathogenesis of Joint Disease in the Mucopolysaccharidoses.** *C.M. Simonaro<sup>1</sup>, M.E. Haskins<sup>2</sup>, E.H. Schuchman<sup>1</sup>*. 1) Dept Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Dept of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Mucopolysaccharidosis Type VI (MPS VI; Maroteaux-Lamy Disease) is the lysosomal storage disorder due to the deficient activity of arylsulfatase B (N-acetylgalactosamine 4-sulfatase). A major feature of this and other MPS disorders is abnormal cartilage and bone development leading to short stature, dysostosis multiplex, and degenerative joint disease. To investigate the pathogenesis of joint disease in MPS VI, primary cultured articular chondrocytes and articular cartilage were examined from MPS VI rats and cats. Increased numbers of apoptotic chondrocytes were identified by TUNEL staining and anti-PARP immunocytochemistry in several joints from the MPS animals. Cultured chondrocytes from these animals also released more nitric oxide and TNF- $\alpha$  into the culture media than normal cells. Notably, dermatan sulfate induced nitric oxide release from normal articular chondrocytes to a degree greater or equal to LPS, a structurally related, pro-apoptotic polysaccharide. Marked proteoglycan and collagen depletion also was observed in the MPS epiphyses by Safranin-O and/or Alcian Blue staining. A model of MPS VI joint pathology is proposed in which dermatan sulfate accumulation leads to an enhanced propensity for chondrocyte cell death, either as a direct effect of the glycosaminoglycan itself or due to generalized lysosomal dysfunction. In turn, the homeostasis of the MPS cartilage matrix is abnormal and the matrix becomes depleted of proteoglycans and collagen. This leads to abnormal articular cartilage function and degenerative joint disease, which is exacerbated by the abnormal mechanical stress placed on the MPS joints and the release of inflammatory cytokines.

**Conditional transgenic over expression of  $\alpha$ -L-Iduronidase.** *C.S. Russell<sup>1</sup>, C.G Lobe<sup>4</sup>, A. Borowski<sup>2</sup>, S.M Gibson<sup>1</sup>, K.E Yip<sup>1</sup>, A. Nagy<sup>3</sup>, F.R. Jirik<sup>2</sup>, L.A. Clarke<sup>1</sup>.* 1) Dept Medical Genetics, Univ British Columbia, Vancouver, Canada; 2) Center for molecular Medicine and Therapeutics, Univ British Columbia, Vancouver, Canada; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada; 4) Cancer Research Division, Sunnybrook Health Science Center, Toronto, Canada.

MPS I is an autosomal recessive genetic disorder resulting from deficiency of alpha-L-iduronidase. MPS I presents as a clinical spectrum of disease ranging from severe skeletal disease, mental degeneration, cardiomyopathy, and death in the first decade (Hurler Syndrome), to milder forms of MPS I with minimal skeletal disease, normal mentation, and viability into adulthood (Scheie syndrome). Treatment options for MPS I include bone marrow transplantation and most recently enzyme replacement therapy (ERT). We have attempted to generate transgenic mice that over express human IDUA for use in BMT and other transplantation studies. Over expression of IDUA appears to be lethal to the developing embryo. Four rounds of pronuclear injection with either myeloid specific (CD11B promoter/enhancer) or ubiquitously expressing (Beta actin promoter/CMV enhancer) transgene constructs produced no transgenic offspring. An approach using embryonic stem cells was employed, one of the lines lead to generation of chimeric animals. We have interpreted this data to suggest that over expression of IDUA severely impairs early embryogenesis. To circumvent this problem we have used a conditional transgene expression approach to generate transgenic ES lines. This conditional expression is mediated by expression of CRE recombinase. Using the expanding repertoire of transgenic mice that express CRE recombinase, we hope to be able to regulate where and when IDUA will be over expressed. This will allow for further studies related to the lethal effects of IDUA over expression as well as the provision of an over expressing murine line for therapeutic studies. The effect of over expression of IDUA indicates that care may need to be taken in relation to the appropriate dose of IDUA for ERT or gene therapy trials.



**Identification and characterization of disease-causing mutations in Type II sialidosis patients.** *S. Pattison*<sup>1</sup>, *C.A. Rupa*<sup>2</sup>, *S.A. Igdoura*<sup>1,3</sup>. 1) Dept. of Biology, McMaster University, Hamilton, Ontario, Canada; 2) Division of Clinical Biochemistry, University of Western Ontario, London, Canada; 3) Dept. of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario,.

Sialidosis is a disease of hereditary sialidase deficiency with various clinical phenotypes. The most severe form, type II (congenital) sialidosis is characterized by hydrops fetalis, hepatosplenomegaly, severe psychomotor retardation and death usually before 1 year of age. Our objective is to identify mutations that cause disease, and to investigate the impact of these mutations on disease phenotype. We report the identification of 5 novel disease-causing mutations from three type II sialidosis patients. Using sialidase specific intron-based primers, PCR of patient derived genomic DNA was performed. Mutations were determined by sequencing and confirmed by restriction enzyme diagnostic. One homozygous nonsense mutation produced a premature stop codon in exon 1. The other four are missense mutation in exons one, four and five. We examined how each of the mutations exert their pathogenic effect by impairing the folding of the sialidase enzyme, or disturbing the assembly of b-galactosidase, cathepsin A and sialidase (GCS) complex. The impact of mutations on enzyme activity toward natural substrates such as GM3 was also assessed in determining disease phenotype, particularly when differences in tissue sialoglycoconjugate accumulations between type II patients are considered. The expression studies of mutant sialidase cDNAs in human primary cell lines provided insights into structurally sensitive protein domains that may be involved in enzyme activity and intercellular localization. Genotype/phenotype correlation of the sialidase mutations will provide a better understanding of the molecular pathology of the disease.

**Urinary glycosaminoglycan excretion quantified by an automated semi-micro method in specimens conveniently transported from around the globe.** *C.B. Whitley, R. Spielman, G. Herro, S. Severson.* Gene Therapy Program, Univ Minnesota Medical Sch, Minneapolis, MN.

Current and future treatments for children with mucopolysaccharidosis diseases require early, presymptomatic diagnosis, yet existing diagnostic methods to quantitate urinary glycosaminoglycan (GAG) are labor-intensive, and thus not applicable for newborn screening. Direct and rapid quantification of GAG excretion with 1,9 dimethylmethylene blue (DMB) is applicable to small volumes of urine collected, dried, and mailed on a paper matrix. To determine if this assay could be automated, a robotic instrument (Biomek 1000) was programmed to accomplish the procedure; the pilot method simultaneously determines GAG and creatinine concentrations in 10 specimens. Each analyte is measured in 4 dilutions thus increasing the operating range to cover a broad spectrum of normal and pathologic levels. Samples and reagents are mixed in 96-well tray format in approximately 20 minutes, and densitometric measurements are recorded in less than 60 seconds. Optical density measurements are electronically transmitted to a desktop computer to select optimal dilutions, identify values above or below the level of reliability, make calculations, and print reports. This automated method was applied to 255 specimens from 101 subjects representing each of the MPS diseases specifically, types I (n=126), II (47), III (48), IV (17), VI (14), VII (3). This method discriminated pathologic elevations of GAG excretion of MPS patients particularly when multiple specimens are available. In contrast to other lysosomal storage disorders, a patient with fucosidosis was found to have markedly elevated levels. Automation of the direct DMB method provides the key technology necessary for newborn screening for MPS diseases.

**MODELING DIFFERENTIAL BINDING OF NF-kB P50 TO A CYP2D6 PROMOTER VARIANT BY INFORMATION THEORY.** *I. Hurwitz, S. Svojanovsky, S. Leeder, P. Rogan.* Clinical Pharmacology, Children's Mercy Hospital, Kansas City, MO.

We determined the functional consequence of the C-1496G promoter variant that has been reported to modulate CYP2D6 activity *in vivo*. *In vitro*, transient transfection assays with the C variant exhibited 4-fold lower activity than -1496G. This SNP falls within an "NF-kB-like" binding motif. EMSAs demonstrate that NF-kB p50/p65 heterodimers do not bind to this region of the CYP2D6 promoter, whereas binding of p50 homodimers to -1496C is ~10-fold greater than to -1496G. These results were analyzed with information theory-based ( $R_i$ , in bits) models of NF-kB binding sites. Model weight matrices derived from published EMSA studies distinguish p50 from p65 homodimers and from p50/p65 heterodimer binding sites, based on their respective average information contents and nucleotide weights at each position. The p50/p65 model ( $R_{\text{sequence}}$ , average information = 11.45 bits) was validated by EMSA with nuclear extracts containing p50/p65 heterodimers and a series of probes with single nucleotide mutations within the commercially available optimal NF-kB binding motif. This sequence (5'-AGTTGAGGGGACTTTCCCAGGC-3') contains a strong 13.3 bit site that is weakened to 5.3 bits (equivalent to  $3^{256}$ -fold decreased binding) in the corresponding commercially available negative control probe (...GGCGACTTTC...). The -1496C allele contains a weak p50/p65 site (-1495 to -1508;  $R_i = 3.3$  bits) that is abolished ( $R_i < 0$ ) in the G variant. These alleles each also contain p50 homodimer binding sites on opposite strands; however, the C allele is predicted to bind with greater affinity (3.5 vs. 2.7 bits;  $3^{1.6}$  fold difference). Given the role of p50/p50 as a negative regulator of gene expression, the higher CYP2D6 activity observed for the -1496G allele may be due to reduced binding and repression by NF-kB p50 homodimers.

*Supported by grant ES10855-01 from NIEHS.*

**Cerebral hypometabolism detected by positron emission tomography (PET) in a patient with complex glycerol kinase deficiency and mental retardation.** *K.M. Dipple<sup>1</sup>, K.M. Venisnik<sup>2</sup>, D.H.S. Silverman<sup>2</sup>, J. Czerin<sup>2</sup>, E.R.B. McCabe<sup>1</sup>.* 1) Department of Pediatrics; 2) Department of Molecular and Medical Pharmacology, Univ California Los Angeles, Los Angeles, CA.

Complex glycerol kinase deficiency (GKD) is due to deletions of the glycerol kinase gene on Xp21 and the classical phenotype involves metabolic acidosis and central nervous system decompensation. In addition, many of the patients with GKD have mental retardation (MR) and seizures. To better understand the MR in these patients, we performed PET using fluoro-deoxyglucose (FDG) to assess glucose metabolism in the brain of a patient with complex GKD. This patient has a deletion involving part of IL1RAPL1, all of DAX1 and GK, and part of DMD (Niakan et al., abstract this meeting). PET has not previously been used to assess the regional brain metabolism in GKD patients. The patient was injected with FDG, and glucose and radioactivity were measured in arterial blood throughout the scan to allow molar quantitation of glucose uptake into brain tissue using compartmental modeling. Region of interest (ROI) analysis was performed by individualized region delineation and by standardized regions drawn on a template to which the patients scan was spatially transformed. Absolute quantitative values of glucose utilization, as well as internally normalized values, were compared to corresponding measurements in control subjects. Both visual interpretation and ROI analyses demonstrated relative and absolute hypometabolism in the parietal, temporal, and basal ganglia regions compared to normal individuals. There was hypermetabolism in the sensorimotor regions, and normal levels of metabolism in the thalamus, cerebellum, and remaining cortex. This is the first study on brain glucose metabolism in a patient with GKD with mental retardation. Parietal and temporal hypometabolism has been noted in several neurodegenerative diseases producing cognitive impairment and reflects a decrease in function of associative cortex. PET in patients with differing breakpoints will help us understand the neurobiological significance of the changes observed and their relationship to the genes in this region.

**Are type 2 and type 3 Gaucher disease a continuum? An intermediate phenotype.** *O. Goker-Alpan<sup>1</sup>, J.K. Park<sup>1</sup>, R. Schiffmann<sup>2</sup>, E. Orvisky<sup>1</sup>, B.K. Stubblefield<sup>1</sup>, N. Tayebi<sup>1</sup>, E. Sidransky<sup>1</sup>.* 1) NSB/NIMH/NIH, Bethesda, MD; 2) DMMP/NINDS/NIH, Bethesda, MD.

Gaucher disease is the inherited deficiency of lysosomal glucocerebrosidase which results in the deposition of glucosylcerebroside in various organs. The disease is classified into three major groups depending on whether the nervous system is involved (type 2 and 3) or not (type 1). The distinction between type 2 and type 3 has been made based upon the age and rate of progression of symptoms, with type 2 being considered acute and type 3 chronic. However, we have identified and genotyped 9 children with neuronopathic Gaucher disease who appear to have an intermediate phenotype. The children were all diagnosed after age 6 months and lived more than one year, but shared acute and rapidly progressive neurologic deterioration. Most died between age 2 and 4 years. Five of the children had been considered type 3 and four type 2, although there was an overlap in their age at diagnosis and their level of residual enzyme activity. Six of the children received enzyme replacement therapy, which had little effect on the progression of the neurologic manifestations. No genotype was common to all of these patients, although most had at least one allele with mutation L444P. Interestingly, 2 patients shared genotype L444P/G202R, but one was considered a type 2 patient and the second a type 3 patient. Thus, it appears that neuronopathic Gaucher disease may actually be a continuum with this intermediate phenotype contributing to the difficulty in distinguishing between type 2 and type 3 Gaucher disease.

**Evaluation of patients with X-linked adrenoleukodystrophy using next generation internet.** *M.L. Ingeholm<sup>1</sup>, B.A. Levine<sup>1</sup>, F. Eichler<sup>2</sup>, G. Raymond<sup>2</sup>, H.W. Moser<sup>2</sup>, G. Jimenez-Sanchez<sup>3,2</sup>.* 1) ISIS Center, Georgetown University Hospital, Washington, DC; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

X-linked adrenoleukodystrophy (X-ALD) affects mainly the nervous system white matter, adrenal cortex and testis. It has an estimated frequency of 1:21,000 in the male population. We have developed a multicenter therapeutic trial in which patients from over 15 countries will participate, including most Latin American countries. Magnetic resonance imaging (MRI) permits early detection of nervous system damage and is a sensitive indicator of disease progression. Initial results from an Internet-1 based network between Baltimore and Minneapolis has shown the important benefits of electronically transmitted MRI in the evaluation of patients with X-ALD. Recently, a new generation Internet (NGI) was developed. This version offers significant advantages over the previous generation, including better image quality, since non-compressed images can be transmitted rapidly, and higher transmission speed of complex MRI and MR spectroscopy images, reducing time to seconds rather than hours. This latter feature makes possible a real-time interaction between site of origin and a central reading site, so that image acquisition can be monitored and modified as the image is being produced. A pilot study compared 78 data transfer trials using standard Internet to 83 data transfer trials using NGI. The NGI showed significant advantages in the overall, among which, the mean transmission time of the NGI (242.5 secs) showed to be nearly an order of magnitude faster than the mean transmission time of the standard Internet (2062.5 secs). Our preliminary results suggest that this will be a valuable aid for the evaluation of disease severity and effects of therapeutic interventions in a multicenter therapeutic trial for X-ALD and may have application in other disease states.

**Glu274Lys/Gly309Arg mutation of the tissue-nonspecific alkaline phosphatase gene in neonatal hypophosphatasia associated with convulsions.** *O. Reish*<sup>1,3</sup>, *I. Litmanovitz*<sup>2,3</sup>, *T. Dolfin*<sup>2,3</sup>, *S. Arnon*<sup>2,3</sup>, *R. Regev*<sup>2,3</sup>, *M. Yamazaki*<sup>4</sup>, *K. Ozono*<sup>4</sup>. 1) Genetic Inst, Assaf Harofeh Medical Ctr, Zerifin, Israel; 2) Department of Neonatology, Meir Medical Center, Kfar Saba, Israel; 3) Sackler School of Medicine Tel-Aviv University, Israel; 4) Department of Environmental Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan.

We describe a patient diagnosed with lethal perinatal hypophosphatasia with a unique clinical presentation of convulsions that responded to vitamin B6. Genomic DNA sequence analysis of the tissue-nonspecific alkaline phosphatase (TNSALP) gene revealed two missense mutations: a G to A transition resulting in a Glu to Lys at codon 274 (E274K), and a G to C transversion resulting in a Gly to Arg at codon 309 (G309R). The first mutation was maternally transmitted and was previously characterized as a moderate one, while the latter was paternally transmitted and has not been previously reported. Phenotype/genotype correlation suggests that G309R is a deleterious mutation that may lead to seizures and a lethal outcome, as was demonstrated in our patient.

**Prosaposin Deficient Mice: Threshold Rescue and Lack of *In Vivo* GrossMorphologic Effects of The Saposin C "Neurotogenic" Region.** Y. Sun, X. Qi, E. Ponce, B. Quinn, G.A. Grabowski. Dept Human Genetics, Children's Hosp Research Fndn, Cincinnati, OH.

Prosaposin, a multifunctional protein, is a precursor of four glycoprotein activators (saposins) for lysosomal hydrolases. The intact prosaposin has lipid transfer properties *in vitro* as well as neurotrophic effects *ex vivo* and *in vivo* when exogenously supplied. Using a transgene (Tg)/knockout (PS<sup>-/-</sup>) strategy, transgenic mice were created to contain the full-length normal (wt) or a mutated (CBC) mouse prosaposin cDNA driven by the PGK (3-phosphoglycerate kinase) promoter. The mutated prosaposin cDNA has the "neurotrophic" region of saposin C replaced by the homologous sequence from saposin B. *In vitro* and *ex vivo* this "saposin CBC" did not promote neurite outgrowth whereas saposin C did. In PS<sup>-/-</sup>;Tg mice the highest levels of transgene expression were consistently in central nerve system (CNS). No major reversal of pathology was appreciated in the visceral tissues of PS<sup>-/-</sup>;Tg (wt or CBC). Compared to PS<sup>-/-</sup> mice, these mice bearing either transgene had delayed onset of neurologic signs and significant extension of life span (up to 5-fold) that were dependent on the level of Tg expression. Similarly, the rate of progression of neuropathology (neuronal storage of glycosphingolipids, hypomyelination, Purkinje cell loss and lipids accumulation) was less in mice with higher levels of Tg expression. For all PS<sup>-/-</sup>; Tg mice neuropathological abnormalities substantially preceded the onset and progression of neurologic abnormalities. PS<sup>-/-</sup>;Tg-CBC had partial rescue but normal brain and spinal cord neuronal migration. These studies show an *in vivo* threshold effect of prosaposin expression and neuropathologic abnormalities. Also, very low steady state levels of prosaposin expression are needed to significantly improve, but not eliminate, the CNS phenotype. Finally, these studies do not support the presence of a significant gross trophic effect of saposin C on CNS development.



Program Nr: 1731 from the 2001 ASHG Annual Meeting

**Modulator Genes in Lesch Nyhan Disease: Microarray Studies.** *D.W. Smith, L. DeGregorio, T. Friedmann.*  
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The mechanisms by which mutations in the hypoxanthine guanine phosphoribosyltransferase (HPRT) gene in humans leads to the devastating neurological phenotype of the Lesch Nyhan syndrome are not well understood. It seems likely that the immediate cause for the neurological dysfunction involves developmental damage to dopaminergic pathways in the brain, but the biochemical and molecular connections between purine pathways affected by HPRT and the dopamine systems are not well characterized. The hypothesis underlying these studies is that the primary defect in HPRT expression results in altered expression of genes that in turn modulate the dopamine pathway. To search for genes and families of genes whose expression is altered in HPRT deficiency, we are using microarray methods to analyze gene expression in the affected brain regions in wild type and HPRT-deficient mice over the post-natal period during which the dopamine defect is established. We shall describe technical aspects of the microarray approach to this disorder and present a description of some candidate modulator genes. These studies were supported by grant HD39250 from the National Institute for Child Health and Human Development and a grant from the Lesch Nyhan Syndrome Children's Research Foundation.

**Cloning and partial characterization of human Agmatinase - concrete evidence for an alternate pathway for polyamine biosynthesis in mammals.** *R.K. Iyer<sup>1,2</sup>, J.H Kim<sup>1</sup>, R.M. Tsoa<sup>3</sup>, W.W. Grody<sup>1,2,4</sup>, S.D. Cederbaum<sup>2,3,4</sup>.* 1) Department of Pathology & Laboratory Medicine, University of California, School of Medicine, Los Angeles, CA; 2) The Mental Retardation Research Center, University of California, School of Medicine, Los Angeles, CA; 3) Department of Psychiatry, University of California, School of Medicine, Los Angeles, CA; 4) Department of Pediatrics, University of California, School of Medicine, Los Angeles, CA.

Polyamines (Spermine and Spermidine) are crucial participants in DNA replication, transcription and protein synthesis, among other functions, and are absolutely essential for cell growth and differentiation of normal cells as well as the proliferation of cancer cells. In mammalian cells Arginine is converted to putrescine, the diamine precursor of polyamines, by the coordinated action of Arginase and ODC; whereas, in *Escherichia coli* and plants, putrescine is synthesized by the enzymes Arginine decarboxylase (ADC) and Agmatinase. Until recently, neither agmatine nor ADC, the enzyme that synthesizes it or Agmatinase the enzyme that is responsible for its metabolism and the synthesis of putrescine were known to exist in man or other mammals. We describe here the cloning and partial characterization of the human Agmatinase gene and the tissue distribution of its transcription product. Human Agmatinase contains 351 amino acid residues and has a calculated molecular weight of 37,688 kD. It has 56% homology to *E. coli* Agmatinase and 42% to human Arginases I and II, sharing highly conserved substrate binding domains. The identification of mammalian Agmatinase supports the possibility of an alternative pathway of arginine metabolism whose products such as agmatine and polyamines can exert powerful and pleiotropic effects in cell growth and function. Possession of this alternate route may help to explain continued polyamine synthesis, and growth in some cancers in which the classic pathway is not present, or is blocked by specific inhibitors.

**Transcriptional regulation mechanisms of holocarboxylase synthetase (HLCS) and biotin dependent carboxylases in multiple carboxylase deficiency (MCD).** *S. Solorzano Vargas, D. Pacheco Alvarez, A. Leon-Del-Rio.* Department of Molecular Biology and Biotechnology, Universidad Nacional Autonoma de Mexico, Mexico, DF, Mexico.

Holocarboxylase Synthetase (HLCS) catalyzes the biotinylation and activation of three mitochondrial and two cytoplasmic biotin dependent carboxylases in humans. Inherited deficiency of HLCS activity causes a disorder known as multiple carboxylase deficiency (MCD), characterized by a blockage of carbohydrates, lipids and amino acid metabolism. MCD is potentially lethal if not treated promptly with pharmacological concentrations of biotin. It has been shown recently that biotin may be involved in other cellular processes in the cells. In this work we show that biotin activates the transcription of Holocarboxylase Synthetase, Acetyl Coa Carboxylase I, and Propionyl CoA Carboxylase. We present here that transcription regulation by biotin is mediated by activation of the soluble form of guanylate cyclase (sGC). Activation of sGC requires a functional HLCS since cells from patients with MCD do not show activation of these genes in response to biotin. These results suggest that the active form of biotin responsible for transcriptional activation is the HLCS biotinylation derivative 5-AMP-biotinyl. Our observations may help to explain the phenotype of patients with MCD. Some of the clinical and biochemical manifestations of the disease cannot be attributed to the individual carboxylase deficiencies and may be the result of a transcription regulation impairment on these patients.

**Photosensitivity in patients with the Smith-Lemli-Opitz Syndrome (SLOS).** *E.R. Elias<sup>1</sup>, I.E. Kochevar<sup>2</sup>, C.R. Taylor<sup>2</sup>.* 1) Special Care Clinic, Children's Hosp, Box 365, Denver, CO; 2) Wellman Laboratories of Photomedicine, Mass Gen'l Hosp, Boston, MA.

Patients with SLOS have an inborn error in the cholesterol metabolic pathway, resulting in severe cholesterol deficiency, and accumulation of the cholesterol precursor, 7-dehydrocholesterol (7-DHC). About one third of SLOS patients manifest severe photosensitivity. A study was designed to evaluate the mechanism of this photosensitivity, by exposing SLOS patients to UV light, and by studying fibroblasts exposed to UV light in the laboratory.

Eight children (ages 2-17 years) with biochemically confirmed SLOS, and pretreatment cholesterol levels ranging from 20-124 mg/dl, were treated with timed exposures to both UVA and UVB light. The reaction was graded by a trained dermatologist to establish the minimum erythema dose (MED), or lowest amount of light exposure necessary to produce an erythematous response. Although only three of the eight patients had shown clinical photosensitivity, all eight had an exaggerated response to timed UVA exposure. The response to UVB was normal in all patients. Treatment with cholesterol reduced clinical photosensitivity, but did not normalize the response to UVA exposure.

Normal fibroblasts were incubated in delipidized medium with AY9944, an inhibitor of the reductase enzyme that is defective in SLOS. The cells were then treated with either 7-DHC or a solvent control, and exposed to varying doses of UVA (2.5-30 J/cm<sup>2</sup>). Cytotoxicity was assessed, and PGE2 release (a mediator of UV-induced inflammation) was measured. Cytotoxicity and PEG2 release were greater in the 7-DHC treated cells. This response was inhibited by the antioxidant, N-acetylcysteine.

These results suggest that the photosensitivity seen in patients with SLOS is caused by UVA light, and may be at least partially due to altered UVA-induced oxidative processes involving 7-DHC in cell membranes. Treatment with cholesterol can reduce clinical photosensitivity in SLOS patients.

**Evidence that *rab27a* mutations are associated with neurological involvement, not simply the hemophagocytic syndrome, in patients with Griscelli syndrome.** *D.L. Fitzpatrick*<sup>1</sup>, *M. Huizing*<sup>1</sup>, *Y. Anikster*<sup>1</sup>, *H. Hurvitz*<sup>2</sup>, *A. Klar*<sup>2</sup>, *E. Gross-Kieselstein*<sup>2</sup>, *W.A. Gahl*<sup>1</sup>. 1) HDB, NICHD, NIH, Bethesda, MD; 2) Ped, Bikur Cholim, Jerusalem.

Griscelli syndrome (GS) is a rare autosomal recessive disorder characterized by pigmentary dilution of the skin and silvery gray hair due to pigment clumping. GS patients have been separated into two subgroups on the basis of clinical and molecular findings. GS1 is defined by severe neurologic impairment and mutations in the *myoVa* gene, coding for myosin Va, a protein functioning with actin in the intracellular movement of vesicles. GS2 is characterized by immune abnormalities and the hemophagocytic syndrome which leads to death in the absence of bone marrow transplantation. GS2 patients have mutations the *rab27a* gene, which codes for a small GTPase involved in vesicle trafficking. Both *myoVa* (mutant mouse: *dilute*) and *rab27a* (mutant mouse: *ashen*) are on chromosome 15q21, < 1.6cM apart. In vitro, *rab27a*-deficient T cells exhibit reduced cytotoxicity and impaired cytolytic granule exocytosis, processes required for immune homeostasis. In vivo, all GS patients with mutations in *rab27a* have developed the hemophagocytic syndrome. However, no GS patient with *rab27a* mutations has manifested neurological features. Our patient, a member of a highly consanguineous Moslem-Arab family with 3 other affected individuals, had silver-gray hair but no tendency to infections. He did suffer from progressive neurological impairment which proved fatal at 10 years of age. Therefore, we screened our patient for mutations in *myoVa*. No mutations were found on cDNA sequencing and a northern blot demonstrated the expected length and signal size for *myoVa*. We next examined the *rab27a* gene. A northern blot of the patient's mRNA, using *rab27a* as a probe, gave no signal. Repeated attempts to amplify the 6 exons of *rab27a* on genomic DNA failed, while two flanking genes (*Loc51187* and *Pigb*) were well-amplified in our patient. These data point to a genomic deletion in the *rab27a* gene of our patient. We propose that a large deletion in *rab27a* may cause neurological involvement in our GS patient, and perhaps in others as well.

**SEQUENCE ANALYSIS OF THE GLUCOSE 6-PHOSPHATE TRANSLOCASE GENE IN PATIENTS WITH GLYCOGENOSIS TYPE IB.** *T. Podskarbi*<sup>1</sup>, *Y.S. Shin*<sup>2</sup>, *A.R. Janecke*<sup>3</sup>. 1) Molec Gen & Metabolism Lab, Munich, Germany; 2) University Childrens Hospital, Munich, Germany; 3) Institute of Human Genetics, Innsbruck, Austria.

Glycogen storage disease type Ib (GSD Ib), characterized by hypoglycemia, hepatomegaly, neutropenia, generalized convulsion and neutrophil dysfunction among others is caused by deficiency of glucose 6-phosphate translocase (G6PT) which catalyses the transport of G6P from the cytoplasm into the endoplasmic reticulum. We report here sequence analysis of the G6PT gene in 15 Caucasian patients whose diagnosis of GSD Ib was secured biochemically either using liver biopsy or analysing glucose transport in PMN cells. All these patients revealed biallelic mutations in the G6PT gene which include five novel mutations. Most of the mutations were frameshift yielding aberrant proteins and others revealed two stopcodon mutations, one large intragenic deletion and missense mutations. The common mutations in exon 8, 1211-2delCT and G339C comprised about 53% of disease alleles. Clinical symptoms of all patients in this study are compatible to those of GSD Ib including neutropenia. No correlation between the genotype and the phenotype could be observed. These data indicate that the molecular analysis of the G6PT gene is an effective mean for the non-invasive and efficient diagnosis GSD Ib.

Program Nr: 1737 from the 2001 ASHG Annual Meeting

**Mutation analysis for mucopolysaccharidosis disorders.** *L. Peterson, E.L. Aronovich, D. Pan, C.B. Whitley.* Gene Therapy Center, University of Minnesota, Minneapolis, MN.

Molecular genetic diagnosis of mucopolysaccharidosis (MPS) disorders is important for a number of clinical applications. Such discrimination of pseudodeficiency states, genotype-phenotype correlation (e.g., early differentiation of relatively attenuated forms of a condition, from more "severe" or classic outcomes). Methods refined over the past decade have allowed us to accomplish complete sequencing of the coding region of several genes, thus establishing a database of common and rare mutations, as well as polymorphisms. Such relatively complete sequence analysis is also necessary to accomplish the experimental procedure known as preimplantation genetic diagnosis (PGD). For PGD, the causative mutations are identified in the proband, and also in both parents. With this information, PGD providers have the information needed to design mutation-specific PCR assays applicable to material derived from the fertilized ovum thus allowing implantation of an unaffected embryo. Although this laboratory has assisted in mutation analysis for 3 families who have contemplated this procedure, we are unaware a pregnancy "at risk" for MPS that has been conceived by this means.

**Succinyl CoA-3-keto Transferase (SCOT) deficiency in a Panamenian patient.** *C.S. Canton<sup>1</sup>, R. Garcia<sup>2</sup>, M. Pasquali<sup>3</sup>, R.H. Sing<sup>3</sup>, K.M. Gibson<sup>4</sup>, N. Longo<sup>3</sup>.* 1) Dept Genetics, Complejo Hospitalario Metropolitano de la Caja de Seguro Social Panama, M.D; 2) Dept Pediatrics, CHMCSS Panama, M.D; 3) Dept Genetics, Emory University, M.D; 4) Bioch Genet Lab, Oregon Health Sciences, University, PhD, FACMG.

Succinyl CoA acetoacetate Transferase is a mitochondrial enzyme which forwards the transfer of Coenzyme A from Succinyl CoA to acetoacetate. Subsequently, mitochondrial acetoacetyl-CoA thiolase converts acetoacetyl to acetylCoA, which can be oxidized in the Krebs cycle. This deficiency is a rare autosomal recessive disorder. We present a female patient with young consanguineous parents. She initially presented at 8 months of age with tachypnea, vomiting, and lethargy. Laboratory testing indicated severe metabolic acidosis with low CO<sub>2</sub> (<5 mEq/L), pH of 6.98, hypokalemia (1.4-2 mEq/L), with elevated anion gap (22-27 mEq/L). Serum ammonia and liver function tests were normal. Urine analysis indicated a pH of 8, negative glucose, and very abundant ketones. There was no evidence of hypoglycemia. Dialysis normalized the acidosis and the anion gap. However, acidosis reappeared when dialysis was discontinued. Biochemical genetic testing indicated abnormal urine organic acids with excess ketones without abnormal metabolites or excess lactic acid on two separate occasions while the child was stable (not acidotic). Ketonuria increased during the acute attack, and very mild lactic aciduria became evident. Plasma and urine amino acids were normal, excluding generalized Fanconi syndrome. A skin biopsy was obtained and the results of enzyme assay on fibroblasts of the patient were absent succinyl CoA: 3-oxoacid CoA transferase (SCOT) activity (normal 4.1-8.1 nmol/min/mg protein). These results confirmed a diagnosis of SCOT deficiency. The treatment consists in a diet poor in fat (25 percent of total calories) and enriched in protein and carbohydrates. The effect of this diet on the anion gap was substantial. At the present she has 2 years and 11 months, psychomotor and physical development are in normal limits. The report is important because this inborn error of the metabolism is very rare (low frequency) and the good neurologic state of the patient is the result of early diagnosis and treatment.



**Gaucher disease and parkinsonism: clinical, molecular and neuropathological findings.** *E. Sidransky*<sup>1</sup>, *R. Schiffmann*<sup>2</sup>, *N. Tayebi*<sup>1</sup>, *D. Krasnewich*<sup>1</sup>, *B.K. Stubblefield*<sup>1</sup>, *A.O. Vortmeyer*<sup>3</sup>, *K. Wong*<sup>4</sup>. 1) NSB/NIMH/NIH, Bethesda, MD; 2) DMMB/NINDS/NIH, Bethesda, MD; 3) SNB/NINDS/NIH, Bethesda, MD; 4) AFIP, Washington, DC.

Among the many phenotypes associated with Gaucher disease, the inherited deficiency of glucocerebrosidase (GC), are reports of patients with parkinsonian symptoms. These patients have an early onset, treatment-refractory form of parkinsonism and often have mild Gaucher manifestations. Sequencing of the GC gene in 12 of such patients revealed no shared genotype and no mutations in the gene for parkin were identified. One patient, studied in great detail, was first diagnosed with Gaucher disease at age 19 because of mild anemia, and was splenectomized at age 34. Her genotype was D409H/L444P, but in addition she carried a duplication resulting from a recombination between the gene and pseudogene for metaxin. She developed a tremor at age 42, followed by rapid deterioration of her gait, and an unsuccessful pallidotomy was performed at age 47. She had impaired horizontal eye movements, tremors, and developed myoclonic jerks, but few systemic manifestations of Gaucher disease. Her neurological symptoms and cognition deteriorated dramatically despite enzyme replacement therapy, and she died at age 52 from aspiration pneumonia. At autopsy, the neuropathological findings were dramatic. She had marked neurodegeneration with demyelination and loss of neurons involving the hippocampus, substantia nigra, dentate and cerebellar Purkinje cell layers. Moreover, there were multiple classic brain-stem-type Lewy bodies, inclusions typically seen in Parkinson disease, found in the hippocampus, pyramidal neurons, substantia nigra and other midbrain structures including the red and oculomotor nuclei which were immunoreactive with anti-ubiquitin and anti-synuclein. However, cortical-type Lewy bodies were not identified in the cerebral cortex. Clusters of Gaucher cells were seen in the leptomeninges although intraparenchymal Gaucher cells were absent. The specific pattern of hippocampal and midbrain involvement observed in this case may provide insight into the pathogenesis of this unique Gaucher phenotype.

**Towards cloning the copper toxicosis gene in Bedlington terriers.** *B. van de Sluis*<sup>1</sup>, *J. Rothuizen*<sup>2</sup>, *P.L. Pearson*<sup>1</sup>, *B.A. van Oost*<sup>2</sup>, *C. Wijmenga*<sup>1</sup>. 1) Department of Medical Genetics, UMC, Utrecht, Utrecht, The Netherlands; 2) Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, 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. A well-regulated copper metabolism is required to ensure a cellular process for copper homeostasis. Only two genetic defects of copper metabolism in man, Menkes and Wilsons diseases have been described. A copper overload disorder, copper toxicosis (CT), is also seen in the Bedlington terrier. CT in Bedlington terriers is an autosomal recessive disorder characterized by an accumulation of copper in the liver leading to chronic hepatitis and, ultimately, cirrhosis. Recently, we assigned the CT locus in Bedlington terriers to the canine chromosome region CFA 10q26, which is homologous to HSA 2p13-21. A radiation hybrid map of the CFA 10q21-26 region was constructed containing 13 DNA markers, 8 genes and 3 ESTs. Moreover, a BAC contig covering the CT region has been finished to facilitate positional cloning of the CT gene. Using homozygosity mapping, the CT locus has been confined to a region less than 5 cR3000 estimated to be 1 Mb, which is entirely contained in a BAC contig. Currently we are in the process of shotgun sequencing the CT-candidate region, in order to obtain a transcript and comparative map of this region. In addition, the draft sequence data of HSA2p13-21 will complement the search for coding sequences. So far, 7 genes from HSA2p13-21 could be mapped back to the CT BAC contig. Sample sequencing revealed 5 additional genes. These genes are currently screened for mutations in affected Bedlington terriers. Isolating a gene for CT will be an important addition to the limited knowledge of the genetic regulation of copper metabolism in mammals.

**A novel, autosomal-recessive form of cardioskeletal myopathy and neutropenia with iron deficiency and Parkinsonism.** *D.G. Brooks<sup>1</sup>, G. Lech<sup>2</sup>, K. VandenBorne<sup>3</sup>, R.B. Wilson<sup>4</sup>, D. Stambolian<sup>5</sup>, B. Chance<sup>2</sup>, R.I. Kelley<sup>6</sup>.* 1) Medical Genetics, Univ. of Pennsylvania, Philadelphia, PA; 2) Dept of Biochemistry and Biophysics; 3) Dept of Physiology; 4) Dept of Pathology; 5) Depts of Genetics and Ophthalmology,; 6) Kennedy-Krieger Institute, Johns Hopkins University, Baltimore, MD.

X-linked cardioskeletal myopathy with neutropenia, and 3-methylglutaconic (3MGC) aciduria (MIM302060), or Barth syndrome (BTHS), is caused by mutations in the Xq28-linked gene, G4.5. G4.5 encodes a fatty acyl transferase-like protein with a probable role in synthesis of cardiolipin, a mitochondrial phospholipid (PL) that is very low in BTHS patients. We report here a non-X-linked form of Barth syndrome with CNS abnormalities and abnormal PL metabolism. Two males and a female born to consanguineous parents were studied. The boys presented with myopathy and neurological regression at ages 5 and 2 years and had increased urinary levels of 3MGC and citric acid cycle intermediates, neutropenia, anemia, and a Parkinsonian tremor. After a 6 month old sister presented with severe dilated cardiomyopathy both older brothers were found to have mild dilated cardiomyopathy and therefore all features of BTHS. Linkage to the G4.5 locus was excluded defining a BTHS variant. Investigating the iron-resistant microcytic anemia revealed increased levels of soluble and lymphoblast transferrin receptor, suggesting intrinsic cellular iron deficiency. Because of evident mitochondrial disease, the children were treated with coenzyme Q and vitamin E, which stopped neurological regression. Later addition of lipoic acid caused improved growth. All three supplements are known to protect cardiolipin from free radical damage. Two Xq28-linked BTHS patients had normal 31P MR muscle spectroscopy whereas two variant-BTHS patients had absent phosphodiester peak which is composed largely of the PL metabolites, glycerophosphorylethanolamine and glycerophosphorylcholine. Infrared spectroscopy showed prolonged recovery time from exercise-induced desaturation in one boy. We speculate that this apparently autosomal recessive BTHS variant is caused by disordered PL metabolism.

**DNA Analysis of Puerto Rican Patients with Albinism Demonstrates that the HPS1 16 bp Duplication Does Not Cause the Majority of Cases.** *A.E. Maldonado<sup>1</sup>, P.J. Santiago-Borrero<sup>2</sup>, J.Y. Rentá<sup>1</sup>, Y. Rodríguez<sup>1,2</sup>, L. Del Fierro<sup>1</sup>, D. Muñoz-Acaba<sup>1</sup>, I. Ortíz<sup>2</sup>, E. Rivera-Caragol<sup>2</sup>, M. Castro<sup>1</sup>, J. Flores<sup>1</sup>, C.L. Cadilla<sup>1</sup>.* 1) Dept. of Biochemistry, UPR School of Medicine, P.O. Box 365067, San Juan, PR 00936-5067; 2) Dept. of Pediatrics, UPR School of Medicine, San Juan, PR.

Albinism is commonly found in the island of Puerto Rico (PR). Previous studies have concluded that the most common form of albinism in PR is the Hermansky Pudlak Syndrome (HPS). Most PR HPS patients examined have a frameshift mutation consisting of a 16 bp duplication in exon 15 of the HPS1 gene, discovered by Spritz and collaborators in 1996. Locus heterogeneity for HPS in PR has been reported in a small number of cases. We have screened 117 Puerto Rican patients with albinism for the HPS1 16 bp duplication and found that only 47% of the patients were homozygous for the duplication, 47% were negative, and 6% were heterozygous for the HPS1 PR mutation. The carrier frequency of HPS in Northwest Puerto Rico was estimated in 1990 to be 1:25, and the prevalence of HPS 1:1800. These estimates were made when no molecular diagnostic test was available. We have preliminary evidence from DNA analysis of PR newborns that the HPS1 16 bp duplication is not as frequently found in the general and Northwest PR populations as previously thought. Out of 326 PR newborns, only five were carriers of the duplication mutation, and only 2/147 came from the Northwest PR region. We have examined HPS1 duplication-negative albino patients for tyrosinase mutations by SSCP and DNA sequencing. We found 5 out of 55 duplication-negative patients homozygous for the G47D mutation in exon 1 of the TYR gene. Some of the duplication-negative patients have evidence of a bleeding diathesis by clinical history, and the results of hematological tests such as platelet aggregation studies, bleeding time and coagulation factor levels. We conclude that the PR HPS1 16 bp duplication accounts for less than half of the albinism cases in PR, and the G47D tyrosinase mutation only accounts for 9% of the albinism cases among HPS1 duplication-negative patients. This study was supported in part by NIH-NIGMS grants S06-GM08224, R25-GM61838 and RCMI grant NCRR G12-RR08051.

**Recombinant adenoviruses mediate doxycycline regulable expression of glycerol kinase in culture.** *K. Nagano, J.J. Chang, Y.-H. Zhang, K.M. Dipple, E.R.B. McCabe.* Department of Pediatrics, UCLA School of Medicine, and Mattel Children's Hospital at UCLA, Los Angeles, CA.

Isolated X-linked glycerol kinase deficiency (GKD) may be either symptomatic or asymptomatic, and we have previously shown that GK genotype does not predict phenotype. A mouse model of GKD will permit improved understanding of pathogenesis and the influence of modifier genes on phenotype. The early death of affected murine males has interfered with characterization of pathogenesis. The purpose of this work was to develop doxycycline regulable adenoviral constructs with the eventual goal of using wild type (wt) and mutant GK expression to rescue and to investigate mice with GKD. We constructed E1/E3 deleted adenoviral vectors (Adeno-X: CLONTECH, Palo Alto, CA) with wt and mutant GKs under control of a tetracycline (tet) response element (TRE) for use in concert with the tet transactivator (tTA) (Adeno-X Tet-Off: CLONTECH). Mutations from symptomatic patients including R413X, R405Q, and D440V, and from asymptomatic patients including A305V and Q438R. We infected HeLa, HepG2, Cos7 and A549 cells, measured GK activity by a radiochemical assay of lysed cells two days after infection, and observed expression in all four cell lines. In HeLa cells, expression required the presence of both Adeno-X Tet-Off and Adeno-X TRE-GK, and was reduced in a dose-dependent manner to negligible GK activity (<1% with MOI of 10) by culture with 10ng/ml doxycycline. Similar to observations in Cos7 cells after transfection with mutant plasmid constructs, the symptomatic R405Q mutation had the highest residual activity when expressed in HeLa cells, again showing an absence of correlation between genotype and phenotype. We conclude that wt and mutant adenoviral GK constructs are expressed in cell culture in a Tet-Off system, and are downregulated effectively by doxycycline. We speculate that in vivo investigations with these constructs in a GKD mouse model will permit rescue and regulable GK expression to investigate pathogenesis and influences on phenotype.

**L-cells lack the ability to convert desmosterol to cholesterol due to a defective *dhsr24* gene.** *P.J. Wightman*<sup>1</sup>, *N. McGill*<sup>1</sup>, *H.R. Waterham*<sup>2</sup>, *D.R. Fitzpatrick*<sup>1</sup>. 1) MRC Human Genetics Unit, Edinburgh, Midlothian, United Kingdom; 2) Departments of Paediatrics and Clinical Chemistry, Division Emma Children's Hospital, Academic Medical Center, University of Amsterdam, The Netherlands.

L-cells are a mouse fibroblasts cell line that can be transfected easily with exogenous DNA and are, thus, widely used for gene expression studies. L-cells exhibit disordered cholesterol biosynthesis which results in the accumulation of desmosterol as the major sterol when grown in the absence of cholesterol. In the 1970's the desmosterol-to-cholesterol converting enzyme (DCE, OMIM 125650) was mapped to human chromosome 20 by complementation using L-cell somatic cell hybrids. Recently human DCE has been shown to be a 3 $\beta$ -hydroxysterol D<sup>24</sup>-reductase now named *DHSR24* and mutations in this gene have been found in two patients with desmosterolosis, an autosomal recessive inherited disorder of cholesterol biosynthesis that results in severe congenital and skeletal anomalies (Waterham et.al. submitted). We have subsequently identified the mouse *dhsr24* gene by homology to the human cDNA sequence. We have isolated mouse genomic BAC clones which contain the entire coding region and have shown the genomic structure to consist of 9 exons and 8 introns. Amplification of each *dhsr24* exon suggested that the first exon was deleted in L-cells. We have stably transformed L-cells using mammalian expression constructs containing the mouse and human *DHSR24* cDNAs, transfection of the expression vector alone and a mutated form of the cDNA were used as negative control. Cells were then grown in media containing delipidated serum. Sterol extraction was performed and the extracts analysed by HPLC. This revealed that the wild type mouse and human *DHSR24* cDNAs restored desmosterol to cholesterol conversion, whereas no restoration was seen with the negative controls. This confirms that the mouse *dhsr24* gene defect is responsible for the desmosterol accumulation in L-cells and are thus a good model for studying the cellular defects in desmosterolosis.

**Rapid Mutation Scanning of the Glucocerebrosidase Gene using Denaturing High Performance Liquid**

**Chromatography.** *P.E. McAndrew<sup>1</sup>, P. Bauer<sup>2</sup>, M. Noyer-Weidner<sup>3</sup>, A. Rolfs<sup>2</sup>, M.A. Marino<sup>1</sup>.* 1) Applied Genomics & Molecular Genetics, Transgenomic Inc, Gaithersburg, MD; 2) Department of Neurology, University of Rostock, Rostock, Germany; 3) Transgenomic Ltd., Berlin, Germany.

Gaucher disease is the most common lysosomal storage disease. The basic defect is a deficiency of glucocerebrosidase, an enzyme required for the lysosomal degradation of glycolipids. Type 1 Gaucher is the most common of the 3 clinical subtypes and is associated with hepatosplenomegaly, anemia, nosebleeds, and bones which break easily. The disorder occurs in all ethnic groups, but has the highest prevalence in the Ashkenazi Jewish population. Type 2 is much less prevalent but is associated with severe neurological symptoms and is often fatal by the age of 18 months. Type 3 Gaucher is similar to Type 2, but the disease progression is less rapid and has a later age of onset. A genetic test to detect five common mutations has ~97% sensitivity in the Ashkenazi Jewish population, and ~70% sensitivity in the general population. An efficient, high-throughput method to screen all Glucocerebrosidase gene (GCB) exons would increase the sensitivity of genetic testing and provide further information about the genotype/phenotype relationships for this disorder. In addition, Type I Gaucher patients with less common mutations who would benefit from the highly effective enzyme replacement therapy can be identified.

We developed denaturing high-performance liquid chromatography (dHPLC) methods to screen all GCB exons using the WAVE® Nucleic Acid Fragment Analysis System. DHPLC uses an ion-pair reversed-phase column to separate heteroduplex molecules from homoduplex fragments in the presence of any base changes, and has become a method of choice for mutation screening. We analyzed a panel of Gaucher patients with common mutations and demonstrate that dHPLC is an efficient method to rapidly screen the GCB gene for known mutations, and we are currently extending our analysis to screen for less common gene alterations which cause Gaucher disease.

**Functional characterization of MCC mutations associated to methylcrotonylglycinuria.** L.R. Desviat<sup>1</sup>, C. Perez-Cerda<sup>1</sup>, J. Esparza-Gordillo<sup>2</sup>, B. Perez<sup>1</sup>, P. Rodriguez-Pombo<sup>1</sup>, J.M. Rodriguez<sup>2</sup>, S. Rodriguez de Cordoba<sup>2</sup>, M.A. Peñalva<sup>2</sup>, M. Ugarte<sup>1</sup>. 1) Centro Biología Molecular, Univ Autónoma, Madrid, Spain; 2) Centro de Investigaciones Biológicas, CSIC, Madrid, Spain.

Methylcrotonylglycinuria (MCG; OMIM 210200) is an autosomal recessive inherited human disorder caused by the deficiency of 3-methylcrotonyl-CoA carboxylase (MCC), involved in leucine catabolism. MCC is a heteromeric enzyme composed of a and b subunits, encoded by the genes *MCCA* and *MCCB*, respectively, which we recently characterized at the cDNA and genomic level. A total of two *MCCA* mutations and 4 *MCCB* mutations were identified in seven patients. Two novel mutations (exon 3 skipping and Q372P in the *MCCA* gene) have now been identified in two additional MCG patients. We have expressed the missense changes in the *MCCA* and *MCCB* genes showing they are clearly pathogenic as they cause reduction or loss of enzyme function. MCC activity was found to be restored to normal levels only when both wild-type *MCCA* and *MCCB* cDNAs are cotransfected in the mutant cell lines, suggesting that coordinated overexpression of both cDNAs is necessary to fully correct the enzyme deficiency. In the *MCCA* gene, the M325R mutation showed low activity (7% of controls) while the residual activity of R385S was undetectable. Both missense mutations C167R and A218T in the *MCCB* gene result in very low levels of residual activity (<2%). No clear genotype-phenotype correlation is evident in this disease newly characterized at the molecular level.



**Effect of *PCCB* gene mutations on the heteromeric and homomeric assembly of propionyl-CoA carboxylase.** S. Muro, B. Perez, L.R. Desviat, R. Rodriguez-Pombo, C. Perez-Cerda, M. Ugarte. Centro de Biología Molecular, Universidad Autónoma de Madrid, Madrid, Spain.

Propionic Acidemia is an inherited metabolic disorder caused by deficiency of propionyl-CoA carboxylase, composed of a-PCC and b-PCC subunits (encoded by genes *PCCA* and *PCCB*) that have been associated to a number of mutations responsible for this disease. After the analysis of the patients' genotypes, the functional characterization of the mutations is an important research area, in order to improve the knowledge of the genotype-phenotype correlations. In the present study 12 *PCCB* gene mutations were analyzed for their involvement in a-b heteromeric and b-b homomeric assembly. For this purpose we performed two-hybrid assay in mammalian COS-7 cells using different cultivation temperatures to distinguish between mutations directly involved in interaction and those probably affecting polypeptide folding and consequently the correct assembly. Mutations R512C, L519P, W531X and N536D, located at the carboxyl-terminal end of the *PCCB* gene, were found to inhibit a-b heteromeric and/or the b-b homomeric interaction independently of the cultivation temperature, reflecting their primary effect on the assembly. Two changes A497V and R165Q did not affect either heteromeric or homomeric assembly. The remaining mutations (R67S, S106R, G131D, R165W, E168K and G198D), located in the amino-terminal region of the b-polypeptide, resulted in normal interaction levels only when expressed at the lower temperature, suggesting that these changes could be considered as folding defects. From these results and the clinical manifestations associated to patients bearing the mutations described above, several genotype-phenotype correlations may be established.

**A Deletion-Insertion Mutation in the Phosphomannomutase 2 Gene in 2 African American Siblings with Congenital Disorders of Glycosylation Type-Ia(CDG-Ia).** *E. Orvisky<sup>1</sup>, N. Tayebi<sup>1</sup>, D. Andrews<sup>1</sup>, J. Park<sup>1</sup>, J. McReynolds<sup>2</sup>, E. Sidransky<sup>1</sup>, D. Krasnewich<sup>3</sup>.* 1) NIH/NIMH, Bethesda, MD; 2) Nemours Children's Clinic, Orlando, FL; 3) NIH/NHGRI, Bethesda, MD.

Congenital Disorders of Glycosylation (CDG) are a group of metabolic disorders with multisystemic involvement characterized by abnormalities in the N-glycosylation of proteins. The most common form, CDG-Ia, resulting from mutations in the gene encoding the enzyme phosphomannomutase2 (PMM2), manifests with severe abnormalities in psychomotor development and multisystemic involvement. While these disorders is panethnic, we present the first cases of CDG-Ia identified in an African American family with two affected sisters. The proband had failure to thrive in infancy, hypotonia, ataxia, cerebellar hypoplasia, and developmental delay. On examination, she also exhibited strabismus, inverted nipples and atypical perineal fat distribution, all features characteristic of CDG-Ia. Direct sequencing demonstrated that the patient had a unique genotype, T237M/c.565-571delAGAGT ins GTGGATTTC. The novel deletion-insertion mutation, which was confirmed by subcloning, introduces a stop codon 11 amino acids downstream from the site of the deletion. The presence of this deletion-insertion mutation at a cDNA position 565 suggests that this site in the PMM2 gene may be a hotspot for recombination.

**Phenylketonuria:genotype-phenotype correlations based on expressed activity of 18 mutant proteins.** *B. Perez, A. Pey, A. Gamez, L.R. Desviat, M. Ugarte.* Centro Biologia Molecular, Universidad Autonoma, Madrid Spain.

Phenylketonuria (PKU) results from the deficiency of phenylalanine hydroxylase (PAH), that catalyses the hydroxylation of phenylalanine to tyrosine. After the identification of more than 400 different PAH gene mutations worldwide, the present research aim is to elucidate their pathologic role in order to gain further insight in the genotype-phenotype correlations. When analysed in the context of the PAH three-dimensional structure, only a minority of the mutations affect catalytic residues, and consistent with these observations, recent data point to defective folding and subsequent aggregation/degradation as predominant disease mechanism for several missense mutations. In this work we use a combined approach of expression in a prokaryotic system with co-expression of chaperonins, in eukaryotic cells at different temperatures and pulse-chase experiments after in vitro synthesis to characterize and confirm structural consequences for 18 PKU mutations localized in the regulatory and catalytic domains of the protein. Four mutations were found to abolish the specific activity in all conditions, the remaining are clear folding mutations causing altered oligomerization, reduced stability, and accelerated degradation although, in addition, some of them affect residues probably involved in regulation. The results derived provide an experimental framework to define the mutation severity relating genotype to phenotype, and allowing to explain the observed inconsistencies for some mutations.

**Characterization of the Human Glycerol Kinase Promoter.** *S.V. Stepanian, K.M. Dipple, E.R.B. McCabe.* Dept Pediatrics, UCLA School of Medicine, Los Angeles, CA.

Isolated glycerol kinase deficiency (GKD) is an X-linked inborn error of metabolism characterized by hyperglycerolemia and glyceroluria which is either symptomatic (episodic metabolic and CNS decompensation) or asymptomatic (pseudo-hypertriglyceridemia). We have been unable to correlate genotype with phenotype in isolated GKD and hypothesize that phenotypic variability might be due to factors influencing GK expression. To test this hypothesis, we characterized the GK promoter, identified potential transcription factors, and analyzed promoter regions for their influences on expression. Computer analysis of the promoter region identified potential HNF-4 consensus binding sites. HNF-4 is involved in the expression of liver-specific genes as well as genes involved in carbohydrate and fat metabolism. Plasmid constructs containing 100, 250, 500, 750, 1500, and 2000 bp (relative to the translation start site) of the GK 5' UTR controlling the expression of a luciferase reporter gene (e.g., GK100LUC) were transiently transfected into human hepatocellular carcinoma (HepG2) cells. Luciferase activity was determined and normalized to beta-galactosidase activity. Three independent experiments showed that GK100LUC was sufficient to activate transcription. GK250LUC and GK500LUC consistently had higher activity than GK100LUC and GK250LUC, respectively, suggesting that transcriptional activators bind between -101 to -250 and -251 to -500 bp. Interestingly, there is an HNF-4 consensus binding site between -433 and -451 bp. GK750LUC, GK1500LUC, and GK2000LUC had less activity than the smaller constructs suggesting that transcriptional repressors may bind between -501 and -2000 bp. These results suggest that the binding of transcription factors, such as HNF-4, may be important for GK expression. Identification of these transcription factors will permit us to examine possible interindividual variations in their response elements in the GK 5' UTR or in their levels of expression. These investigations will allow us to examine the hypothesis that influences on transcriptional activity may mediate phenotypic variability in isolated GKD.

**Clinical features, response to treatment, prognosis, and molecular characterization in Korean patients with inherited urea cycle defects.** *H.W. Yoo, G.W. Kim, E.J. Seo.* Medical Genetics Clinic & Laboratory, Asan Medical Center, Ulsan University College of Medicine, 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. Five well characterized diseases have been described, resulting from an enzymatic defect in the biosynthesis of one of the normally expressed enzyme. This presentation will focus on two representative diseases; ornithine transcarbamylase(OTC) deficiency and citrullinemia(argininosuccinate synthetase deficiency). Ornithine transcarbamylase deficiency is one of the most common inborn error of urea cycle, which is inherited in X-linked manner. We identified 16 different mutations in 20 unrelated Korean patients with OTC deficiency; L9X, R26Q, T44I, R92X, G100R, R141Q, G195R, M205T, H214Y, D249G, R277W, F281S, 853 delC, R320X, V323M and 10 bp del at nt. 796-805. These mutations occur at well conserved nucleotide sequences across species or CpG hot spot. The L9X and R26X lead to the disruption of leader sequences, required for directing mitochondrial localization of the OTC precursor. Their phenotypes are severe, and neonatal onset. The G100R, R277W and V323M mutations were uniquely identified in patients with late onset OTC deficiency. The other genotypes are associated with neonatal onset. Out of 20 patients with OTC deficiency, only 6 patients are alive; two were liver transplanted, and normal in growth and development at 2, 4 years after transplantation respectively. Citrullinemia is an autosomal recessive disease, caused by the mutations in the argininosuccinate synthetase(ASS) gene. We identified in 3 major mutations in 10 unrelated Korean patients with citrullinemia; G324S, IVS6-2 A to G, and 67 bp ins at nt 1125-1126. Among these, the 67 base pair insertion mutation is novel. The allele frequency of each mutation is; G324S(45%), IVS6-2 A to G(40%), and 67 base pair insertion(10%). All patients are diagnosed at neonatal or infantile age. Interestingly, two patients presented with stroke like episode. All patients except one are surviving on dietary management.

**Pheno-genotypic studies of childhood neuronal ceroid lipofuscinoses.** *N. Zhong*<sup>1,2,4</sup>, *D. Moroziewicz*<sup>1,2</sup>, *W. Ju*<sup>1,2</sup>, *K.E. Wisniewski*<sup>3,4</sup>, *S.S. Brooks*<sup>2</sup>, *W.T. Brown*<sup>2</sup>. 1) Molecular Neurogenetic Diagnostic Laboratory, NYS Inst Basic Res, Staten Island, NY; 2) Dept. Human Genetics, NYS Institute for Basic Res, Staten Island, NY; 3) Dept. Neuropathology, NYS Institute for Basic Res, Staten Island, NY; 4) Dept. Neurology, SUNY-Health Science Center at Brooklyn, Brooklyn, NY.

Neuronal ceroid lipofuscinoses (NCLs) are a group of common childhood neuronal degenerative disorders. A common feature of the rapid progression of psychomotor and cognitive deterioration characterizes the NCLs, however, affected individuals differ in clinical presentation. To better characterize phenotypes and genotypes and to obtain a better understanding of NCL pathogenesis, we analyzed age-at-onset, initial symptoms/signs, and clinical progressive course; electron microscope (EM) examination for lipofuscin storage profiles; lysosomal enzyme assays for PPT1 and TPP1; and molecular analyses for gene mutations. A total of 392 clinically diagnosed subjects affected with NCL were studied. Among these, INCL accounts for 20.6%, LINCL for 40.6%, and JNCL for 38.9%. The initial symptoms/signs of seizures, speech/learning disabilities, vision loss, motor dysfunction, dementia, and behavioral changes were determined to be 11.1, 33.3, 36.1, 19.4, 0, and 0% in INCL; 39.4, 33.8, 14.1, 5.6, 2.8, and 4.2% in LINCL; and 5.9, 22.1, 57.4, 1.5, 7.4, and 5.9% in JNCL. The predominant EM profile in the INCL was pure GR (51.2%), followed by GR+CV/FP (17%) and GR+CV+FP (9.7%). However, FP and FP+CV (59.4%), followed by CV (16.7%), were the common profiles in JNCL and CV (66.2%), followed by CV+FP (16.9%) and CV+FP+GR (13.8%), in LINCL. Mutation analyses showed that common mutations in both alleles usually associate with typical clinical courses, however, uncommon mutations in one or both alleles may result in atypical progression. A small portion of NCL families has been found could not be classified with known NCL variant forms in our studies, which we grouped as NCL9. We propose that NCL9 is underlied by a yet-unidentified gene CLN9. Currently, searching for the gene CLN9 is being undertaken.

**Genotype-Phenotype correlations in cystathionine beta synthase deficiency.** *M.T. Steen<sup>1</sup>, R.H. Singh<sup>1</sup>, M. Pasquali<sup>1</sup>, L.J. Elsas II<sup>1</sup>, W.D. Kruger<sup>2</sup>.* 1) Div Medical Genetics, Emory Univ, Atlanta, GA; 2) Div Population Genetics, Fox Chase Cancer Center, Philadelphia, PA.

We evaluated genotype-phenotype relationships among twelve patients from 11 families with cystathionine beta synthase (CBS) deficiency. Using DNA sequencing, we identified 21 of 22 possible mutant CBS alleles, and characterized both clinical and biochemical phenotypes. Each missense mutation in CBS was engineered into a recombinant *S. cerevisiae* strain containing an expressed human CBS cDNA as its sole source for CBS activity, and bioassayed for the correction of cysteine auxotrophy. Crude cell extracts were then prepared and enzymatic activities were determined. Previously described mutations and their CBS activities (in parentheses, expressed in nanomoles/mg protein/hr) were: wild type (350), G307S (0), I278T (20), V320A (90), T353M (6), and L101P (2). Six novel mutations first identified in this study were: A226T (26), N228S (22), A231L (12), D376N (0), and Q526K (8). One splice site mutation was found (G<sub>736</sub>-1 C). The I101P, V228S, V320A, T353M, and D376N mutations demonstrated clinical B6-nonresponsiveness, while the A226T mutation was B6-responsive. The T353M mutation was found exclusively in each of four American Black patients. Five I278T and five T353M alleles accounted for 45% of the 22 alleles in this patient cohort. We conclude that there is a genotype-phenotype relationship in CBS deficiency.

**NDUFV2 mutation in a case of hypertrophic cardiomyopathy and mitochondrial complex I deficiency.** *P. Benit<sup>1</sup>, M. Corral-Debrinsky<sup>2</sup>, P. de Lonlay<sup>1</sup>, J.P. Issartel<sup>3</sup>, D. Chretien<sup>1</sup>, A. Munnich<sup>1</sup>, P. Rustin<sup>1</sup>, A. Rotig<sup>1</sup>.* 1) Dept Genetics, INSERM U393, Hosp Necker, Paris, France; 2) UMR 8541, Ecole Normale Suprieure, 75005 Paris, France; 3) Laboratoire de BioEnergétique Cellulaire et Pathologique, Dpartement de Biologie Molculaire et Structurale, CEA, 38054 Grenoble Cedex 9, France.

NADH:ubiquinone oxidoreductase (complex I) is the largest complex of the mitochondrial respiratory chain. This complex accounts for most cases of respiratory chain deficiency in human. Only seven mitochondrial DNA genes but more than 35 nuclear genes encode complex I. Among them, the NDUFV2 gene encodes the 24 kDa subunit which contains a Fe-S-binding site. In an attempt to elucidate the molecular bases of complex I deficiency, we studied the NDUFV2 gene in a series of 40 patients with isolated complex I deficiency. The full length NDUFV2 mRNA extracted from cultured skin fibroblasts was reverse transcribed and tested by Denaturing High Performance Liquid Chromatography (DHPLC). Abnormal fragments were then directly sequenced. We identified a NDUFV2 mutation in a patient born to first cousin parents of West African origin. He presented a severe hypertrophic cardiomyopathy, hypotonia, growth retardation and profound isolated complex I deficiency in skeletal muscle. Accordingly, oxidation of malate-pyruvate was decreased in his cultured skin fibroblasts. Three older sibs had previously deceased from cardiomyopathy. Sequencing of NDUFV2 cDNA and genomic sequence revealed a deletion of exon 2 due to a 4 bp deletion in intron 2 (IVS2 120+5D4bp) in the intron splice site. The resulting protein is predicted to lack amino acids 19-40 thus removing part of the mitochondrial targeting signal peptide and its cleavage site. Western blot analysis of mitochondria from cultured skin fibroblasts of the patient revealed that the amount of NDUFV2 protein was decreased to 30-40% as compared to control. This is the first report of a NDUFV2 mutation in a case of complex I deficiency.



**2-Methyl-3-hydroxybutyryl-CoA dehydrogenase: purification of the enzyme, cloning of the cDNA and resolution of the molecular basis of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase deficiency.** *R.J.A. Wanders<sup>1</sup>, R. Ofman<sup>1</sup>, M. Feenstra<sup>1</sup>, J.P.N. Ruiten<sup>1</sup>, J. Zschocke<sup>2</sup>, H.R. Waterham<sup>1</sup>.* 1) Clinical Chemistry, Laboratory for Genetic Metabolic Diseases, Academic Medical Centre, University of Amsterdam, The Netherlands; 2) Universität Kinderklinik, Heidelberg, Germany.

We recently identified a new defect in the isoleucine breakdown pathway, 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD/SCHMAD) deficiency, in a boy showing progressive infantile neurodegeneration. The normal activity of other mitochondrial 3-hydroxyacyl-CoA dehydrogenases including the short-(SCHAD) and long-chain (LCHAD) enzyme, suggested the existence of a distinct 3-hydroxyacyl-CoA dehydrogenase. We now report the purification of this enzyme from bovine liver using several different column chromatography steps. The candidate protein band was excised from the SDS/PAGE gel and subjected to MALDI-TOF mass spectrometry after in gel digestion with trypsin. Screening of several databases resulted in a positive identification. The authenticity of its cDNA was established by expressing this cDNA in *E. coli* followed by enzyme activity assays. Molecular studies in the index patient as well as other patients with MHBD/SCHMAD-deficiency revealed distinct mutations.

**Two-Dimensional Electrophoresis, The Best Screening Method for Mucopolysaccharidoses: Comparing the Reliability With Acid Turbidity Test and Berry Spot Test.** *C. Chuang, S. Lin.* Medical Research Dept, MacKay Memorial Hosp, Taipei Taiwan, Republic of China.

Because of the differences in glycoaminoglycan (GAG) contents and quantities in various mucopolysaccharidoses (MPS), the MPS screening tests including acid turbidity test and Berry spot test are not specific and sensitive enough for the preliminary diagnosis of MPS. A false-negative interpretation resulted in a mistaken diagnosis happens frequently. We analyzed urine samples collected from 33 MPS patients (2 with MPS I, 15 with MPS II, 9 with MPS III, 5 with MPS IV, and 2 with MPS VI) and 24 control children using acid turbidity (qualitative and quantitative) and Berry spot tests, respectively. All the samples were confirmed by the dimethylmethylene blue method (DMB) for GAG quantitation, and two-dimensional electrophoresis (2-D EP) for MPS differential diagnosis. With the exception of MPS I, II, and VI, patients with MPS III and IV showed 44.4% and 60% false-negative results, respectively, in which the concentrations of uronic acid in the urine samples were all less than 1.5 mg/mL and the spot test showed very faint metachromatic pattern. Conclusively, the DMB ratio (GAG mg/mmol creatinine) is not correlated to the results obtained from acid turbidity test and Berry spot test, on the contrary, the latter two have good correlation to each other. Therefore, a misdiagnosis of MPS by using both tests is possible. For an accurate diagnosis of MPS, the 2-D EP method is recommended. The 2-D EP method provides a good separation of GAG contents, high sensitivity, specificity, and efficiency, in addition, a false-negative interpretation of MPS is rarely happened.

**VALIDATION OF GLUCOSE TETRASACCHARIDE AS A BIOMARKER FOR DIAGNOSIS AND MONITORING ENZYME REPLACEMENT THERAPY FOR POMPE DISEASE.** *Y. An, D.S. Millington, P. Kishnani, A. Amalfitano, Y.T. Chen.* Division of Medical Genetics, Department of Pediatrics, Duke Univ Medical Center, Durham, NC.

Glycogen storage disease type II (GSD-II), also known as Pompe disease, results from lysosomal acid  $\alpha$ -glucosidase (GAA) deficiency. Clinically, GSD-II encompasses a range of phenotypes from the infantile form with hypotonia, muscle weakness, cardiac hypertrophy and culminating in early death, to the juvenile and adult-onset forms characterized by progressive respiratory and limb muscle involvements. The objective of this research was to validate a tetraglucose oligomer, Glc1-6Glc1-4Glc1-4Glc, designated (Glc)4, as a biomarker for monitoring the disease state and therapeutic response in GSD-II patients. (Glc)4 levels in blood and urine samples collected from either animal models of GSD-II, or GSD-II patients were measured by a newly developed HPLC test method. Validation for (Glc)4 as a biomarker included measuring the glycogen content and (Glc)4 level in the muscles of GSD-II quails and normal quails. The (Glc)4 levels in GSD-II quail muscles were 0.01-0.02 mg/mg protein. In normal quails, the (Glc)4 levels were undetectable. The ratio of (Glc)4 to total glycogen content in Pompe quail muscles was 2-3%. Moreover, in vitro tests suggested that the (Glc)4 is likely derived from partial digestion of glycogen by neutral glucosidases. The (Glc)4 levels correlated well with the clinical forms of the disease with infantile and early juvenile patients having higher (Glc)4 levels than the later-onset adult patients. In addition, (Glc)4 levels were inversely related to the serum GAA activity levels measured in infantile patients receiving recombinant enzyme therapy. As a further validation of the utility of the (Glc)4 biomarker to monitor GSD-II therapies, we found that the (Glc)4 levels increased in patients that began mounting anti-rhGAA antibody responses, and that the (Glc)4 levels decreased after therapeutic maneuvers were initiated (increased frequency of enzyme infusions, or immune-suppressive strategies). Our results indicate that (Glc)4 is a promising biomarker for monitoring the disease state and therapeutic response in Pompe disease.

**Phenotypic differences between sialidosis and galactosialidosis mouse models.** A. D'Azzo<sup>1</sup>, E. Bonten<sup>1</sup>, L. Mann<sup>1</sup>, N. DeGeest<sup>2</sup>. 1) Department of Genetics, St. Jude Children's Research Hospital, Memphis, TN; 2) Department of Genetics, Katholieke Universiteit, Gasthuisberg Campus, Leuven, Belgium.

Neuraminidase initiates the hydrolysis of sialo-oligosaccharides, -glycolipids and -glycoproteins by removing their terminal sialic acid residues. In humans, primary or secondary deficiency of this enzyme leads to two clinically similar neurodegenerative lysosomal storage disorders, sialidosis and galactosialidosis. Mice nullizygous at the *Neu 1* locus develop clinical abnormalities reminiscent of early onset sialidosis in children, including severe nephropathy, progressive edema, splenomegaly, kyphosis and urinary excretion of sialylated glycoproteins. As observed in patients with these diseases, the sialidosis mouse model share clinical and histopathological features with GS mice. However, we have identified phenotypic abnormalities that are exclusively present in sialidosis mice. Progressive deformity of the spine, high occurrence of early death, age-related extramedullary hematopoiesis, and lack of apparent degeneration of Purkinje cells appear diagnostic for this disease model only. Thus the differences and similarities between the two mouse models may help to better understand the pathophysiology of these diseases in children and to identify more target therapies.

Supported in part by NIH grants GM60950, DK52025, and CA21765, Assisi Foundation of Memphis, and the American Lebanese Syrian Associated Charities (ALSAC).

**Correction of lysosomal PPCA and neuraminidase in mouse deficient macrophages after uptake of recombinant baculovirus-expressed proteins.** *E. Bonten, A. d'Azzo.* Department of Genetics, St. Jude Children's Research Hospital, Memphis, TN.

Galactosialidosis (GS) is a lysosomal storage disorder caused by combined deficiency of protective protein/cathepsin A (PPCA), b-galactosidase, and neuraminidase (neur). Patients develop a multi-systemic disease mostly accompanied by severe neurological deficit and early death. Their clinical symptoms are similar to those of sialidosis patients and have been mainly attributed to the secondary deficiency of neur. The dependence of neur to PPCA for intracellular targeting and activation underscores the need for therapeutic modalities that would supply the patients with both enzymes. Recombinant PPCA precursor is secreted efficiently from normal and over-expressing mammalian cells, and can be taken-up by deficient fibroblasts and other cells via the M6P receptor, correcting the enzyme deficiency. In contrast, neur, although secreted, lacks a functional M6P recognition marker and is internalized poorly by mammalian cells. With the intent to investigate the potential of ERT for the treatment of GS we have used recombinant baculovirus-expressed proteins (BV-neur and BV-PPCA) as source of the corrective enzymes. The recombinant proteins are biologically functional, and can be produced in large quantities at low cost. BV-expressed neur and PPCA were tested in uptake experiments on cultured BM macrophages. Both BV-PPCA and BV-neur, either alone or mixed, were efficiently taken up by PPCA-deficient mouse macrophages, and capable of restoring cathepsin A and neuraminidase activities. Maximum correction of neuraminidase activity (>10 fold control values) was established with a mixture of PPCA and neur. Uptake was blocked by the addition of yeast mannan, indicating that the proteins were taken up via the macrophage-specific mannose receptor. These results set the basis for ERT trials in PPCA and neur deficient mouse models, using BV-derived recombinant proteins. The use of BV-produced proteins may be applicable to other lysosomal disorders.

Supported in part by NIH grants GM60950, DK52025, and CA21765, Assisi Foundation of Memphis, and the American Lebanese Syrian Associated Charities (ALSAC).

**Characterization of a canine model of mucopolysaccharidosis IIIB.** *N.M. Ellinwood<sup>1</sup>, P. Wang<sup>1</sup>, T. Skeen<sup>2</sup>, N. Sharp<sup>2</sup>, M. Cesta<sup>2</sup>, W. Bush<sup>1</sup>, E. Hardam<sup>1</sup>, M.E. Haskins<sup>1</sup>, U. Giger<sup>1</sup>.* 1) Univ. Pennsylvania, Philadelphia, PA; 2) North Carolina St. Univ., Raleigh, NC.

Mucopolysaccharidosis III (MPS III) has been documented in numerous species, including mice (MPS IIIA and IIIB), dogs (MPS IIIA), emus (MPS IIIB), and goats (MPS IIID). We now report on a canine form of MPS IIIB; on the clinical, biochemical and pathologic features, and the preliminary genetic characterization of the canine N-acetyl-a-D-glucosaminidase (NAGLU) locus.

Two 3-year-old Schipperke dogs were evaluated by the Metabolic Screening Laboratory of the Veterinary Hospital of the University of Pennsylvania. The affected dogs had progressive, severe neurological signs consistent with diffuse cerebellar disease. Both animals were confirmed to have heparan sulfaturia. Assays of fibroblast cultures for eleven lysosomal enzymes indicated a deficiency of NAGLU activity, making the diagnosis of MPS IIIB. Pedigree analysis and levels of NAGLU activity of family members supported autosomal recessive inheritance, as expected for MPS IIIB. Due to neurological deterioration, both dogs were euthanized, and complete post-mortem examinations were performed. Biochemical studies of tissues from both animals confirmed a lack of NAGLU activity, storage of sulfated glycosaminoglycans, and secondary elevations of other lysosomal enzymes, including b-glucuronidase and total b-hexosaminidase. Pathology of the brain included severe cerebellar atrophy and lysosomal storage in neurons and perithelial cells.

The use of canine MPS IIIB offers an opportunity to further assess the pathogenesis of MPS IIIB and to evaluate possible therapies. To this end, an obligate male carrier was used to establish a research colony. Initial genetic analysis of the normal canine NAGLU locus has begun with the production of canine specific NAGLU sequence, comprising one third of the predicted canine cDNA. Completed investigations will include the characterization of the entire canine NAGLU locus and confirmation of the causative mutation responsible for canine MPS IIIB.

Supported by NIH Grant RR02512.

**A Fluorescence-Based, High Performance Liquid Chromatographic Assay to Determine Acid Sphingomyelinase Activity and Diagnose Types A and B Niemann-Pick Disease.** *X. He, F. Chen, E.H. Schuchman.* Department of Human Genetics, Mount Sinai School of Medicine, New York, NY.

Acid sphingomyelinase (ASM, sphingomyelin phosphodiesterase, E.C. 3.1.4.12) is the lysosomal enzyme that hydrolyzes sphingomyelin (SPM) to phosphorylcholine and ceramide. An inherited deficiency of ASM activity results in Types A and B Niemann-Pick disease (NPD). In this study we report a new assay method to detect ASM activity and diagnose NPD using the fluorescent substrate, Bodipy C12-SPM (B12SPM, Molecular Probes Inc.), and reverse-phase high performance liquid chromatography (HPLC). The reaction product, Bodipy C12-ceramide (B12Cer), could be clearly and efficiently separated from the substrate (B12SPM) within 4 min using a hydrophilic column (Aquasil C18, Keystone Scientific Inc.). Femtomol quantities of the reaction product, B12Cer, could be detected in as little as 0.1 ml of human plasma, providing a sensitive measure of ASM activity. Mean ASM activity in human plasma from NPD patients (36 pmol/ml/h) was only 2.7% of that in normal plasma (1334 pmol/ml/h), confirming the specificity and diagnostic value of this new assay method. Importantly, mean ASM activity in human plasma from NPD carriers (258.3 pmol/ml/h) was also significantly reduced (19.5% of normal). The ranges of ASM plasma activities in NPD patients (N=19), carriers (N=11), and normal subjects (N=15) were 2.5-97.4, 108-551, and 1030-2124 pmol/ml/h, respectively. Based on these results, we suggest that this fluorescence-based, HPLC assay method is a reliable, rapid, and highly sensitive technique to determine ASM activity, and that plasma is a very reliable and simple source for the accurate diagnosis of NPD patients and carriers based on ASM activity. This new method may also serve as a prototype for assaying various other biologically important sphingomyelinases and may be useful in a wide range of experimental circumstances where the in vitro detection of ASM activity is necessary.

**A first successful bone marrow transplantation case of galactosialidosis.** *J. Hwang<sup>1</sup>, D. Im<sup>1</sup>, J. Lee<sup>1</sup>, E. Kwon<sup>1</sup>, J. Kim<sup>1</sup>, J. Kim<sup>2</sup>, D. Jin<sup>1</sup>.* 1) Pediatrics, Samsung Medical Center, Seoul, Korea; 2) Clinical Pathology, Samsung Medical Center, Seoul, Korea.

Galactosialidosis is a lysosomal storage disease associated with a combined deficiency of b-galactosidase and neuraminidase, secondary to a defect of another lysosomal protein, the protective protein/cathepsin A (PPCA). Although a transgenic mouse model demonstrated a promising outcome of bone marrow transplantation (BMT), there was no reported case of BMT in the literature. Here we report a first successful BMT results in a Korean patient whose enzyme assay confirmed galactosialidosis. The index case of the family was his younger sister who showed typical coarse face, scoliosis, mental retardation, myopia, generalized tonic clonic seizure and cherry red spot. There was a progressive aggravation of her neurologic symptom and eventually she was bed-ridden, and enzyme assay for lysosomal storage disorders was evaluated. The enzyme assay of her fibroblast revealed combined deficiency of b-galactosidase and neuraminidase which confirmed the galactosialidosis. Incidentally, her brother showed the similar morphologic manifestation which prompted us to evaluate him. He also had cherry red spot and abnormal increase of glycosaminoglycan, but there was no hepatosplenomegaly. Several months after diagnosis, he also showed gait disturbance and neurologic deterioration. Thus he was treated with bone marrow transplantation with haploidentical donor, his mother. One hundred days after BMT, b-galactosidase activity in his leukocyte were normalized and his neurologic derangement was stationary. However, his post-BMT course was complicated at 9 months after BMT by severe varicellar infection and he succumbed to pulmonary hemorrhage due to uncontrolled infection. We concluded that his neurologic deterioration was halted before he succumb at least. Here we report a first BMT case of galactosialidosis.



**Mutation analysis of iduronate-2-sulfatase gene in 20 Korean families with mucopolysaccharidosis type II. H.**

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Mucopolysaccharidosis(MPS) II is an X-linked recessive lysosomal storage disorder caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (IDS) because of several different types of mutations in the IDS gene. Two forms of MPS II are distinguishable clinically. A severe form has progressive mental retardation and physical disability and death before age 15 years in most cases. A mild form has a slower progression of somatic deterioration and retention of intelligence. The responsible IDS gene consists of 9 exons and 8 introns structurally. It is sequenced a 2.3-kb cDNA clone coding for the entire sequence of human IDS. The IDS cDNA detected structural alterations or gross deletions of the IDS gene in many of the clinically severe form of MPS II patients studied. In Korea, MPS type II is the most prevalent form among MPS. In our Genetic clinic of Samsung Medical Center, we are following up 30 families of MPS type II. Among these,sequencing analysis with a total 20 families of MPS II was performed in order to identify the primary genetic lesion and to find the novel mutation. Seventeen different mutation of IDS gene were detected.: 1 splicing mutation, 3 missense mutation, 5 nonsense mutation, 6 deletion mutation and 2 insertion mutation. The nine of them were novel and unique.: 1 novel missense mutation: Leu259Pro, 2 novel nonsense mutations: Glu177X(GAA->TAA) and Lys213X (AAA->TAA), 4 novel deletion mutations: 121-123bp(c41,CTC), 69-88bp(c23-c30, TGC^GTGGCctcgcgatccgaaacgcagCCAAC), c263(GCC->-CC), 1042-1049(c348-c350, GCC^AAAtacagcaaTTTGAT), 2 novel insertion mutations: c8G(CGA->GCGA), c228(CCC->CCCA). We can expect that new mutation detection contributes to the level of heterogeneity for in MPS II.

**Mutation analysis of the acid ceramidase gene in Japanese patients with Farber disease.** *K. Inui, T. Muramatsu, N. Sakai, Y. Yamada, H. Tsukamoto.* Dept Pediatrics, Fac Medicine, Osaka Univ, Osaka, Osaka, Japan.

Farber disease is a rare lysosomal storage disease, characterized by the accumulation of ceramide in tissues due to the acid ceramidase deficiency. Here we report the identification of three novel mutations in the acid ceramidase gene in two Japanese patients. Patient 1 showed joint problems around 10 months old and she is now emaciated with multiple nodules at 9 years old and mild neurological problems. Patient 2 was the product of consanguineous parents and showed joint contracture around 8 months old. She showed neurological symptoms around 2 years old and died at 6 years old due to respiratory failure. The diagnosis was made by clinically and confirmed by enzymatic assay of acid ceramidase. In molecular analysis using cultured skin fibroblasts, normal mRNA levels were expressed in both patients. By direct sequencing of cDNAs, missense mutations of V97E in exon4 and G235R in exon 9 were detected in patient 1 and 96delV in exon 4 was homozygously identified in patient 2. These mutations were also confirmed in genomic DNAs. Expression of the mutated acid ceramidase cDNAs in COS-1 cells and subsequent determination of acid ceramidase residual enzyme activity demonstrated that each of these mutations was the direct cause of the acid ceramidase deficiency in the respective patients. We also found a new polymorphism V369I in exon 14 in the allele from the mother of the patient 1. Until now only several mutations were reported in Farber disease. These mutations including our new identified mutations show genetically privates in Farber disease.

**Biochemical and molecular analysis of infantile free sialic acid storage disease (ISSD) in a North American child.**

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Our patient was born at 38 weeks of gestation after an uneventful pregnancy. Neither her parents nor grandparents were of Finnish origin. Her growth and development appeared normal at 4 weeks of age. Subsequently, the child showed progressive growth retardation and developmental delay. A seizure disorder was diagnosed at the age of 6 months. Facial features appeared course at 7 months of age. Muscular tone decreased gradually and respiratory difficulties became a major medical issue. The child died at the age of 33 months. Elevated urinary sialic acid was documented by thin layer chromatography at 17 months of age. Fibroblast studies confirmed this finding, with levels of 54 and 89 nmol free sialic acid/mg protein (normal, 1). Most of the free sialic acid (62%) was within the lysosomal, or granular, fraction. Recently, Salla disease and ISSD were shown to be allelic disorders involving mutations in SLC17A5 and, consequently, defective sialin. This lysosomal membrane transporter is responsible for the egress of free sialic acid out of lysosomes. We performed mutation analysis of the SLC17A5 gene using the patient's cDNA and genomic DNA. On the cDNA level, the patient was compound heterozygous for a 148-bp deletion of exon 9 and a 15-bp deletion (del 801-815) in exon 6 of the SLC17A5 gene. The 148-bp deletion (del 1112-1259), which encompassed the entire exon 9, was due to a GA splice site mutation in position 1 of intron 9. In summary, we demonstrate the biochemical diagnosis of ISSD by showing increased free sialic acid within fibroblast lysosomes, and the molecular diagnosis by confirming a new example of compound heterozygosity for SLC17A5 gene mutations in a North American child. The differential diagnosis of developmental delay and growth retardation in early childhood is difficult, and is particularly challenging in infancy. ISSD and Salla disease are important members of the differential diagnosis in children who show developmental delay of postnatal onset.

**The E326K mutation and Gaucher disease: Mutation or Polymorphism?** *M.E. LaMarca<sup>1</sup>, J.K. Park<sup>1</sup>, D. Stone<sup>1</sup>, J.J. MacKenzie<sup>2</sup>, N. Tayebi<sup>1</sup>, E. Sidransky<sup>1</sup>*. 1) NSB, NIMH/NIH, Bethesda, MD; 2) Queens University, Ontario, Canada.

Gaucher disease is caused by mutations in the gene for human glucocerebrosidase, a lysosomal enzyme involved in the intracellular hydrolysis of glucosylceramide. While over 150 different glucocerebrosidase mutations have been identified in patients with Gaucher disease, not all mutations have been fully characterized as being causative. One such mutation is the E326K mutation, which results from a G→A nucleotide substitution at genomic position 6195. There are several cases of patients with Gaucher disease and the E326K mutation; however, in each instance, the E326K change was found as a second mutation on an allele with another glucocerebrosidase mutation. Utilizing PCR screening and restriction digestions of both patients with Gaucher disease and normal controls, we identified the E326K allele in both groups. Of the 216 alleles screened from patients with Gaucher disease, the E326K mutation was detected in 4 alleles (1.9%). Each carried another known glucocerebrosidase mutation. In addition, screening for the E326K mutation among normal controls revealed that 3 alleles among 130 screened (2.3%) also carried the E326K change. In the normal controls with the E326K allele, the glucocerebrosidase gene was completely sequenced but no additional mutations were found. Therefore, we believe that the E326K mutation may not be a causative change and stress that a careful examination of any allele with this mutation should be performed to check for the presence of another glucocerebrosidase mutation.

**Tissue specific expression and regulation of CTNS.** *A. Helip Wooley, J. Thoene.* Hayward Human Genetics Center, Tulane University School of Medicine, New Orleans, LA 70112.

Cystinosis results from defects in the CTNS gene which encodes the lysosomal cystine transporter cystinosin. The mouse homolog of CTNS (Ctns) encodes a protein that is 92.6% similar to human cystinosin. Tissue specific expression of Ctns in the mouse was examined using the mouse Rapid-Scan Gene Expression Panel consisting of 96 well PCR plates of first strand cDNAs in 4 serial dilutions representing 24 different tissues or embryonic stages (Origene). Each cDNA is normalized to contain equivalent amounts of B-actin cDNA, then PCR amplified using primers for either Ctns or B-actin as a control. Ctns is expressed in all tissues and embryonic stages examined, with the highest levels of expression seen in liver, kidney, lactating breast and testis. Upregulation during lactation may reflect increased proteolysis to facilitate milk protein production. Adrenal, the only tissue not found to store cystine in cystinotic humans, had the lowest level of Ctns expression in the panel. Cultured cells treated with cystine di-methylester (CDME) accumulate high concentrations of cystine in their lysosomes. Human embryonic kidney (HEK293) cells were cultured in the presence or absence of 0.2 mM CDME for 1, 3, or 7 days. RNA was harvested on the indicated day and analyzed by northern blot using PCR radio-labeled probes for either CTNS or B-actin. Similar levels of CTNS expression in CDME treated and untreated cells were seen on day 1. On days 3 and 7, however, CTNS message is almost absent in the untreated cells but the CDME treated cells continue to express high levels of CTNS. Levels of B-actin were similar for all samples. This difference in CTNS expression is attributed to the untreated cells entering a stationary phase as they reach confluence with minimal transcription of CTNS, while the CDME treated cells, though also reaching confluence, are stimulated by lysosomal cystine to maintain expression of CTNS at high levels. These studies indicate cystinosin expression is somewhat tissue specific in the mouse and may be influenced by lysosomal cystine content in cultured human cells.

**Subcellular localization and correction of CTNS gene mutations.** *R. Lemons, M.A. Park, A. Helip-Wooley, J. Thoene.* Hayward Human Genetics Center, Tulane University School of Medicine, New Orleans, LA. 70112.

Mutations of the CTNS gene resulting in intermediate or nephropathic cystinosis have been described. A splice site mutation (IVS11+2 T ® C) that eliminates the GYDQL lysosomal targeting sequence of cystinosin yields an intermediate phenotype, but can produce cystine depletion when transfected into CTNS null fibroblasts. The possibility of an alternate lysosomal targeting sequence was evaluated by constructing plasmids with N-terminal GFP fused to either normal CTNS or CTNS with the splice site mutation. The normal CTNS-GFP fusion product was found to co-localize with the lysosomal marker lysosensor red almost exclusively, while the splice site CTNS-GFP fusion product was found in the plasma membrane and cytoplasm as well as lysosomes. This finding was supported by Cherqui, et al who recently described a second, less efficient, lysosomal targeting sequence, YFPQA, in CTNS which is retained in the truncated protein resulting from the splice site mutation. A nonsense mutation in CTNS (753 G ® A) encoding a premature termination codon (PTC) at amino acid 138 (W138X) has been described. Gentamicin is known to induce PTC read-through and hence full length protein production. We have demonstrated that gentamicin treatment (300 mg/ml) leads to depletion of intracellular cystine in cell strains with a premature termination codon, but not in those with large deletions or a deletion leading to a frame shift mutation. Plasmids with GFP as a C-terminal fusion to CTNS or PTC-CTNS were constructed allowing GFP to serve as a reporter. The normal CTNS-GFP fusion co-localized with lysosomes. No significant fluorescence was observed in PTC-CTNS transfected cells in the absence of gentamicin but green fluorescence was demonstrated and shown to co-localize with lysosomes in its presence. These findings were confirmed by immunofluorescence labeling with anti-GFP antibodies. Using GFP fusion proteins we have demonstrated lysosomal targeting of normal CTNS, partial lysosomal targeting of a CTNS splice site mutation using an alternate targeting sequence, and aminoglycoside induced PTC read-through of a PTC-containing CTNS mutation.

**Site-specific recombination of the NPC1-gene in human cells - a model for the analysis of biochemical consequences of different mutations.** *R. Oheim, P. Bauer, R. Knoblich, M. Zschesche, C. Bauer, A. Rolfs.* Dept. of Neurology, University of Rostock, Rostock, M/V.

Niemann Pick Type C (NPC) disease is a rare, autosomal recessive lipid storage disorder involving the nervous system. In at least 95% of all NPC-patients mutations can be identified in NPC1 gene, which encodes for a lysosomally targeted transport protein. NPC is characterized by moderate to severe hepatosplenomegaly, pronounced neonatal jaundice, supranuclear gaze palsy and movement disorders such as ataxy and cognitive impairment. Biochemically, the translocation of LDL-derived unesterified cholesterol from the lysosomes to the Golgi is blocked. In order to study phenotypical consequences of different mutations within the NPC1 gene and to develop a genetically based therapeutic treatment we have produced a knock-out cell-line as NPC-model. For this reason a knock-out vector was constructed, which is based on the pBSbluescript, including two regions of homology to the NPC gene, namely LA (ca. 1,8 kb) and RA (ca. 4,0 kb). These fragments are interrupted through a neomycin-box, which causes the exon-knock-out in the genom of the cells and is necessary for positive selection. For negative selection with ganciclovir the vector was designed with a herpes simplex virus thymidin kinase box 3' from the RA-fragment. The homologous fragments were constructed using long distance PCR and cloned into the vector using restriction enzymes. We chose XbaI and SalI for the LA-fragment and XhoI and BamHI for the RA-fragment. Two different cell lines were transfected by electroporation, first the haploid CHO-KI to avoid problems of generating homozygous knock-out in diploid cells and to control the functionality of the vector and second 1321LN, a human astrocytoma cell line, to establish a NPC1-model related to the nervous system. Genetic and biochemical analyses including microarray studies of the homozygously NPC1 knocked-out cells will follow.

**Site-specific recombination of the glucocerebrosidase-gene in human cells - a model for the analysis of biochemical consequences of different mutations.** *K. Heintze<sup>1</sup>, P. Bauer<sup>1</sup>, R. Knoblich<sup>1</sup>, C. Bauer<sup>1</sup>, M. Horowitz<sup>2</sup>, A. Rolfs<sup>1</sup>.* 1) Dept. of Neurology, Univ. of Rostock, Germany; 2) Dept. of Cell Res. and Immunology, Tel Aviv Univ., Israel.

Gaucher disease (GD) is a lipid storage disorder characterized by impaired activity of the lysosomal enzyme glucocerebrosidase (GBA), which involves the nervous system in a variable manner. Its clinically heterogeneity is attributed to a large number of mutations in the GBA-gene but also depends on the given cellular environment within the neuronal and glial cells or fibroblasts. In a prospective study we have analyzed more than 300 patients for mutations within the GBA-gene by sequencing the entire GBA-gene. The data demonstrate a relative low prevalence of the well known mutations 370Asn>Ser and 444Leu>Pro which account for only 40% of the GD-producing alleles. Additionally, we have identified 37 new mutations, e.g. 809C>T (231Thr>Ile), 827C>T (237Ser>Phe), 882T>G (255His>Glu), 1028A>G (304Tyr>Cys), 1208G>A (364Ser>Asn), 1227C>A (370Asn>stop), 1296G>A (393Trp>stop). In order to study the biochemical crosstalk between those GBA-mutations (444Leu>Pro, 393Trp>stop) found mainly in the neuronopathic forms of the disease and the consequences in the given cell population we underwent a targeted disruption of the GBA-gene. The targeting vector, as a replacement construct, contained two regions of homology genomic fragments (1,5 kb containing exon 1-3 and 4 kb containing exon 5-10) of the GBA-gene on either side of the positive selectable marker neomycin. Furthermore, a negative selectable marker, the herpes simplex virus thymidine kinase, was introduced into the targeting vector outside the region of homology to GBA. The 1,5 kb genomic fragment (left arm, LA) was generated by PCR with oligonucleotides which contains the Restriction-sites SalI (sense Primer) and XbaI (antisense Primer). The 4 kb fragment (right arm, RA) was generated by long-distance PCR and contains XhoI/ClaI. The recombination will be done in human neuronal cell lines in comparison to fibroblasts. A detailed biochemical and genetic analysis of the homozygously GBA-knocked-out cells is followed by micro-array studies.



**Abnormal regulation of genes encoding lysosomal and cytoskeletal proteins in fibroblasts from patients with lysosomal disorders.** *P.R. Hunt*<sup>1</sup>, *C.R. Bouton*<sup>2</sup>, *J. Pevsner*<sup>1,2,3</sup>. 1) Doctoral Program in Human Genetics and Molecular Biology, The Johns Hopkins University, Baltimore, MD; 2) Department of Neuroscience, The Johns Hopkins School of Medicine, Baltimore, MD; 3) Department of Neurology, Kennedy Krieger Institute, Baltimore, MD.

Although individually rare, lysosomal storage disorders share many clinical characteristics, and as a group, have an estimated frequency of about 1 in 5,000 births. Clinical symptoms are progressive, and often include mental disturbance and regression, characteristic facies, enlarged liver and spleen, and respiratory and cardiac problems. While most lysosomal storage disorders are characterized by defects in genes encoding specific lysosomal enzymes, several are characterized by defects in lysosomal or endosomal transport, e.g. mucopolysaccharidosis types II (MLII) and IV (MLIV), Chediak-Higashi Syndrome (CHS), and Niemann Pick Disease Type C (NPC). We hypothesized that, since trafficking to lysosomes is perturbed in these disorders, the diseased cells may respond by differentially regulating the expression of genes encoding lysosomal proteins. By cDNA microarray analysis we measured the expression of 9,000 genes in fibroblasts from patients with MLII, MLIV, CHS, NPC, and in chloroquine-treated fibroblasts in which swollen endosomal compartments are induced. Data analysis employed the DRAGON database to identify changes in 200 groups of functionally related genes. In MLII, MLIV, CHS, and chloroquine-treated fibroblasts, but not in NPC, we found increased expression of genes encoding lysosomal proteins. Surprisingly, we also found a group of genes encoding cytoskeletal elements to be down-regulated in MLIV. Findings are confirmed at the protein level by Western analysis and immunofluorescence microscopy. These studies provide insight into the cellular response to lysosomal disorders.

**A biliary phenotype of Type 1 Gaucher disease(GD): high prevalence of cholesterol gallstones(GS)associated with abnormal lipid metabolism.** *P.K. Mistry<sup>1</sup>, H. Hyogo<sup>2</sup>, K. Lim<sup>1</sup>, W. Lomazow<sup>1</sup>, H. Chung<sup>1</sup>, H. Deng<sup>2</sup>, A. Stancato-Pasik<sup>1</sup>, G. Hermann<sup>1</sup>, S. Wallenstein<sup>1</sup>, D.E. Cohen<sup>2</sup>.* 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Liver Research Center, Albert Einstein School of Medicine, Bronx, NY.

Type 1 GD is due to a recessive deficiency of acid b glucosidase, which leads to widespread accumulation of macrophages laden with lysosomal glucosylceramide. Consistent with the finding of efficient biliary secretion in rats, gcosylceramide does not accumulate in heptocytes of GD patients. Nevertheless, a biliary phenotype of GD has not been described. **AIM:** To assess the prevalence and determinants of GS (asymptomatic GS plus previous cholecystectomy). **METHODS:** The study cohort comprised 322 consecutive patients with type 1 GD,171 females, 151 males, aged <sup>3</sup> 10 yrs (mean 48±17)and 230 had intact spleens while 90 were asplenic. **RESULTS:** Compared to the general population overall prevalence of GS was more than 5-fold higher, increasing from 5% in 10-19 yrs to 67% in >70 yrs age group (mean prevalence 35%). GS were more frequent in asplenic patients compared to those with intact spleen: 53% vs 32%, p<0.001. Patients with GS had higher body mass index(p<0.001) and higher LDL cholesterol(p<0.001) than those without. On multivariate analyses, significant correlates of GS were older age(p<0.001, splenectomy(p<0.001), female sex(p=0.005) and HDL cholesterol(p=0.027). Overall, cholesterol levels and body mass index of GD patients was below 95th percentile. Cholesterol GS were identified in three patients and pigment stones in the other. Biliary lipid composition in biles of 4 GD patients was markedly altered containing high concentration of glucosylceramide(334-1068 mM) compared to normal approximately 1mM(Pentchev et al, BBA, 665,615,1981). GD biles also contained glucosylsphingosine 1.5-4.8mM. **CONCLUSIONS:** Low body mass index, extremely low plasma cholesterol, abnormal biliary lipid secretion associated with marked propensity to cholesterol GS represent a hitherto undescribed metabolic syndrome of GD. We speculate that in GD, glucosylceramides profoundly affect biliary lipid secretion and cholesterol solubility in bile.

**Chiari type 1 malformation in three unrelated patients affected with Fabry disease. P. HALIMI<sup>1</sup>, D.P. GERMAIN<sup>2</sup>.**

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Fabry disease (FD) (OMIM 301500) is an X-linked recessive disease resulting from deficiency of the lysosomal hydrolase alpha-galactosidase A. The enzymatic defect leads to the widespread deposition of uncleaved neutral glycosphingolipids in the plasma, and lysosomes of many cell types, especially in vascular endothelial and smooth muscle cells. Initial clinical signs include skin lesions (angiokeratoma), excruciating acral pain, and benign corneal opacities. Progressive glycosphingolipid deposition in the microvasculature of hemizygous males subsequently leads to ischaemic complications involving kidneys, heart and brain, and to failure of target organs. Among neurological symptoms, strokes and transient ischaemic attacks have been reported. A 29-year-old male patient, with a confirmed diagnosis of FD, was referred to us for evaluation of a sudden episode of dizziness with disequilibrium and diplopia, in agreement with the diagnosis of transient ischaemic attack. Magnetic resonance imaging showed minimal cerebrovascular involvement. In particular, no sign of stroke, vertebral arteries abnormalities, or bulbar infarction was evidenced. However, sagittal MRI revealed Chiari type 1 malformation (CM1). CM1 is a pathological continuum of hindbrain maldevelopments, characterized by downward herniation of the cerebellar tonsils. CM1 is defined as tonsillar herniation of more than 5 mm below foramen magnum. Following the ascertainment of this first patient, we systematically performed cerebral magnetic resonance imaging in a cohort of 15 male patients affected with FD, and identified two additional cases of association between FD and CM1 in two unrelated patients. This survey is the first report on the association between FD and CM1. Our data emphasize that CM1 malformation should be considered in the differential diagnosis of cerebrovascular manifestations of FD. In addition, since both conditions are rare, the association may possibly not be coincidental.

**Cystinotic fibroblasts display aberrant apoptosis.** *M.A. Park, A.H. Wooley, J.G. Thoene.* Human Genetics, Tulane University, New Orleans, LA.

Cystinosis is a lysosomal storage disorder characterized by abnormally high lysosomal cystine levels due to a defect in the lysosomal cystine transporter cystinosin. Patients with the disorder manifest failure to thrive, the renal Fanconi syndrome, progressive renal failure, hypothyroidism, retinopathy and keratopathy. The proximal cause of cell, tissue and organ death is not known, nor is it easily inferred, given that the cystine accumulation remains partitioned in the lysosomal compartment. The lysosomal cysteine and aspartic proteases, cathepsins B and D respectively, are newly characterized effectors in the apoptotic response pathway. Cathepsin D translocates from the lysosomes to the cytosol in response to a pro-apoptotic signal. There it activates pro-apoptotic caspases. Cathepsin B acts as an inhibitor of cathepsin D. Examination of cystine inhibition of purified human hepatic cathepsin B using a fluorogenic substrate found that 10 uM cystine inhibited 0.1uM cathepsin B by 87%. Direct measurement of cathepsin B activity in cystinotic and normal fibroblast lysates showed that the cystinotic cells demonstrated only 65% of normal activity per milligram cell protein, and that this activity was restored to normal by addition of DTT to the lysates. Apoptosis was induced in cystinotic and normal cell lines, and the cells were analyzed by TUNEL or Annexin V staining followed by fluorescence microscopy. Cystinotic fibroblasts (cell line GM00008), were found to undergo apoptosis about 4 - 20 times more readily than normal fibroblasts (GM00010) when microinjected with cytochrome C. A similar response was observed in cystinotic and normal fibroblasts treated with anti-Fas antibodies, thapsigargin, and etoposide. We hypothesize that cystine-induced inhibition of cathepsin B leads to aberrant apoptosis, and may be responsible for inappropriate cell death in cystinosis, leading to the phenotype.

**Patients with Gaucher disease and myoclonic epilepsy.** *J.K. Park<sup>1</sup>, R. Schiffmann<sup>2</sup>, N. Tayebi<sup>1</sup>, E. Orvisky<sup>1</sup>, C. Kaneski<sup>2</sup>, M.E. LaMarca<sup>1</sup>, E. Sidransky<sup>1</sup>.* 1) NSB, NIMH, Bethesda, MD; 2) DMNB, NINDS, Bethesda, MD.

Gaucher disease, resulting from the inherited deficiency of the lysosomal enzyme glucocerebrosidase, is divided into three clinical types based upon the presence and rate of progression of neurological symptoms. Type 1 is the most common form and has no associated neurological symptoms, whereas type 2 presents with acute and progressive neurologic involvement. Type 3, by definition, includes those patients with chronic neurologic involvement who have survived past the first few years of life, but the associated neurological symptoms can be quite variable. One rare subgroup of patients with type 3 Gaucher disease are those that develop myoclonic epilepsy. We evaluated the phenotypes and genotypes of 14 patients with Gaucher disease and myoclonic epilepsy utilizing molecular techniques including PCR, long template PCR, direct sequencing and Southern blot analysis. Generally, this group of patients had severe visceral involvement with the onset of seizures by adolescence. All of the 14 patients had involvement of the horizontal saccadic eye movements. Twelve different genotypes were encountered. Shared alleles included G377S, V394L and N188S, each seen in 2 alleles, and L444P and recNciI, each seen in 4 alleles. Two patients each shared the genotypes V394L/RecNciI and N188S/RecNciI. In addition, another patient had the genotype N188S/c.84insG. Both V394L and N188S are mutations that have previously been described as associated with non-neuronopathic Gaucher disease. As this group of patients with a rare phenotype does not share a specific genotype, the possibility of modifying genes is being considered. Since genotype alone cannot accurately predict phenotype, the focus on patients with unique phenotypes, such as patients with myoclonic epilepsy, may lead to a greater understanding of the molecular complexity encountered in Gaucher disease.

**Gene conversion events in patients with Gaucher disease.** *N. Tayebi, B.K. Stubblefield, M.E. LaMarca, J.K. Park, B.M. Martin, E. Sidransky.* NSB, NIMH, Bethesda, MD.

Gaucher disease is the autosomal recessively inherited deficiency of the lysosomal enzyme glucocerebrosidase (GC). The human GC gene, located on chromosome 1q21, has a pseudogene that shares 96% sequence homology and is located 16kb downstream of the functional gene. The presence of this pseudogene increases the possibility of recombination within this locus. Patients with Gaucher disease are divided into three types, based upon the presence and rate of progression of neurological manifestations: type 1, non-neurologic, type 2, acute neurologic, and type 3, subacute neurologic. We examined the genotypes of patients with Gaucher disease utilizing PCR, long template PCR, direct sequencing, Southern blots and polymorphism analyses. We found 55 alleles carrying a recombination between the GC gene and its pseudogene in 205 patients with Gaucher disease. Thirty-two of the recombinant alleles resulted from gene conversion events, the majority of which occurred within an 830 nucleotide sequence extending from the end of intron 8 to the beginning of exon 10 of the GC gene. Moreover, in the 17 recombinant alleles identified in a subgroup of 32 patients with type 2 Gaucher disease, we found 11 gene conversion alleles. Several possible sites of gene conversion were identified. In one of the gene conversion alleles, the shortest length of homology between the acceptor and donor sequence was only 23 nucleotides. Comparison of the sequences at the identified sites of gene conversion revealed a shared pentamer motif sequence (CACCA) that may be a hot spot for gene conversion. These findings substantiate the premise that the 3' end of the GC gene has a great proclivity for recombination events. Therefore, genotypic analysis of patients with Gaucher disease, especially type 2, should be performed with attention to molecular detail to enable more accurate studies of genotype-phenotype correlation.

**Cholelithiasis in patients with Gaucher disease.** *H. Rosenbaum*<sup>1</sup>, *E. Sidransky*<sup>2</sup>. 1) Dept. of Hematology, Rambam Medical Center and Rappaport Faculty of Medicine, Haifa, Israel; 2) NSB, NIMH, Bethesda, MD.

Gaucher disease, the inherited deficiency of the lysosomal enzyme glucocerebrosidase, manifests with a wide range of clinical features. We noted that a number of adult patients with type 1 Gaucher disease also had gallstones. The clinical records of 65 adult patients evaluated at the National Institutes of Health with type 1 Gaucher disease were reviewed and 19 patients were identified who either had gallstones or a history of a cholecystectomy. Of the 19 patients, six were male. The age at which stones were noted ranged from 19 to 70 years (mean 35 years). Thirteen of the patients had a cholecystectomy performed. Several different factors may contribute to the development of gallstones in these patients. Eleven of the patients were found to have chronic anemia, including 3 with autoimmune hemolytic anemia. Fifteen of the patients underwent splenectomy. To determine whether our findings were specific to our referral population, the medical records of a second series of 80 adult patients of Ashkenazi Jewish ancestry with type 1 Gaucher disease followed in Northern Israel were reviewed. Sixteen of these patients (5 male, 11 female) were also noted to have gallstones. To our knowledge this is the first report of gallbladder involvement in Gaucher disease.

**Gaucher Disease: Creation of point mutations in mouse acid b-glucosidase (GCase).** *Y.H. Xu, T. Leonova, E. Ponce, G.A. Grabowski.* Dept Pediatrics, Div Human Gen, Children's Hosp Medical Ctr, Cincinnati, OH.

Gaucher disease is an autosomal recessively inherited disease caused by the mutations in the GCase locus leading to disruption of the enzyme's structure and function. Toward understanding the molecular bases of Gaucher disease, using the cre-lox system, 5 point mutations (N370S, V394L, D409H, D409V, or D399N) were introduced into mouse GCase to mimic the mutations in human disease with differential phenotypes. The mutations were confirmed by sequencing of both genomic DNA and RT-PCR products. Compared with wild type (WT), the V394L homozygotes (>160 day survival) showed a reduced GCase in liver ( $5.6 \pm 1\%$  of wt), spleen ( $19 \pm 0.6\%$ ) and brain ( $50 \pm 1\%$ ). N370S homozygosity is lethal and these mice died shortly after birth. To study the expression of the mutant GCases *ex vivo*, skin fibroblasts were established from FVB-WT<sup>+/+</sup>, V394L<sup>+/-</sup>, V394L<sup>-/-</sup>, N370S<sup>+/-</sup>, N370S<sup>-/-</sup>, V394L<sup>-/null</sup>, N370S<sup>-/null</sup>, null/null, and Neo-N370S<sup>-/-</sup>. GCase activities in these fibroblasts were 61.2%, 24.4%, 59.8%, 13.5%, 13.4%, 5.8%, 2.4%, and 0.59% (n=2-7) of WT (FVB strain), respectively. In parallel, the tTA-off or rtTA-on system (tetracycline transactivator and human GCase TARGET gene) has been developed for conditional rescue of GCase mutant mice. In the Lap-tTA-off system, GCase expression was increased to 250% in liver and to 200% in spleen in absence vs presence of tetracycline. Studies in these point-mutant and conditionally expressing mice will facilitate understanding of the pathophysiology of Gaucher disease.



**Molecular analysis of GM1-gangliosidosis: identification and characterization of four novel mutations in two Taiwan Chinese patients.** *F.J. Tsai<sup>1,2,3</sup>, C.F. Yang<sup>2,3</sup>, J.Y. Wu<sup>2,3</sup>*. 1) Dept Pediatrics, China Medical Col Hosp, Taichung, Taiwan; 2) Dept Medical Research, China Medical Col Hosp, Taichung, Taiwan; 3) Dept Medical Gen, China Medical Col Hosp, Taichung, Taiwan.

GM1-gangliosidosis is an autosomal recessive lysosomal storage disease caused by a deficiency of acid beta-galactosidase (GLB1; EC3.2.1.23). By use of direct sequencing, mutation screening of the GLB1 gene was performed in two Taiwan Chinese patients with severe form of GM1-gangliosidosis. Patient 1 carries a G-to-T change at nucleotide position 1481 (exon 15) on one allele, which converts glycine into valine (Gly494Val), and a three-nucleotide deletion on the other allele, resulting in an in-frame deletion of one leucine residue (c.945-947del). Patient 2 was characterized to have three different nucleotide changes. She carries a C-to-G change at nucleotide position 304 (exon 3) on one allele, which converts histidine into aspartic acid at residue 102 (His102Asp). She also has a C-to-T transition at nucleotide 902 (exon 8) on the other allele, which converts alanine into valine at residue 301 (Ala301Val). In addition to Ala301Val, this patient also has a G-to-A change at nucleotide position of 67 in exon 1 on the same allele, leading to the substitution of serine for glycine at residue 23 (Gly23Ser). Transient overexpression in COS-1 cells with cDNA encoding Ala301Val, Gly494Val, His102Asp, c.945-947del, Gly23Ser mutant b-galactosidases produced products with activities of 0, 0, 14, 2.9, 94.7 % compared with the cDNA clone for wild-type b-galactosidase. The transient overexpression in COS-1 cells allows us to discriminate mutations affecting catalytic activity from those that did not. In conclusion, we have identified four novel mutations and one polymorphism in two Taiwan Chinese patients with severe form of GM1-gangliosidosis.

**Barriers to successful dietary control among pregnant women with phenylketonuria (PKU).** *A.S. Brown<sup>1</sup>, P. Fernhoff<sup>2</sup>, S. Waisbren<sup>3</sup>, D. Frazier<sup>4</sup>, R. Singh<sup>2</sup>, F. Rohr<sup>3</sup>, J. Morris<sup>1</sup>, A. Kenneson<sup>1</sup>, P. MacDonald<sup>1</sup>, M. Gwinn<sup>1</sup>, M. Honein<sup>1</sup>, S. Rasmussen<sup>1</sup>.* 1) CDC, Atlanta, GA; 2) Emory University, Atlanta, GA; 3) Children's Hospital, Boston, MA; 4) University of North Carolina, Chapel Hill, NC.

Despite the known and preventable risks associated with maternal PKU, affected babies continue to be born, emphasizing the need to identify barriers to successful dietary control among pregnant women with PKU. To investigate these barriers, a questionnaire was used to interview women with PKU known to metabolic disease clinics in three states who were pregnant during 1998-2000. Of 24 women in the study, 22 (92%) followed the phenylalanine-restricted diet during pregnancy; only 8 (33%) initiated the diet prior to pregnancy. Of 22 medical records received, only 12 (55%) indicated control of blood phenylalanine levels prior to 10 weeks gestation. Women who achieved metabolic control after 10 weeks gestation were five times as likely to be younger than 25 years of age (RR= 5.1, 95% CI = 1.4, 18.9) and believe that treatment costs complicated the diet (RR= 5.0, 95% CI = 1.3, 18.6). All of the women expressed confidence in the metabolic clinic staff, but only 8 (33%) perceived their obstetricians were familiar with maternal PKU pregnancy risks. Of 13 women enrolled in state-based assistance programs, 9 (69%) reported proof of pregnancy was required for eligibility. Many women using private insurance reported their insurer was unwilling to pay for medical foods. When the data were stratified according to state of residence, differences were observed in the live birth rate, pre-pregnancy formula use, average travel time to the metabolic clinic, and gestational week when metabolic control was achieved. These findings suggest that women with PKU need more education about the importance of dietary control before conception. Obstetric knowledge of maternal PKU needs further evaluation. Discrepancies should be resolved between maternal PKU medical recommendations and the policies of third party-payers. The disparities in financial assistance and services available to pregnant women with PKU residing in different states should be examined further.

**Evaluating the Dose Response in Maternal Phenylketonuria.** *A.E. Kanaga, J.D. McDonald.* Dept of Biological Sciences, Wichita State University, Wichita, KS.

In the human inborn error of metabolism, phenylketonuria, there is an important maternal effect syndrome; namely maternal phenylketonuria. In this syndrome, a fetus being gestated in the uterus of a phenylketonuria female is damaged due to her metabolic defect. We are using the *BTBR-Pah<sup>enu2</sup>* mouse phenylketonuria model and a synthetic diet (Teklad Research Diet #97152, Harlan-Teklad, Madison, WI) to study the relationship between maternal hyperphenylalaninemia and birth defects among offspring. By varying maternal genotype between mutant and non-mutant and by varying the concentration of phenylalanine in the diet, we can investigate the dose response between maternal blood phenylalanine level and fetal birth defect incidence. A number of differing dietary conditions are used to produce a range of maternal blood phenylalanine levels between normal and classical phenylketonuria levels. To monitor the incidence of birth defects, fetuses are assessed at term for three gross morphometric parameters known to be affected in human maternal phenylketonuria offspring: body weight (BW), crown-to-rump length (CRL), and head circumference (HC). Fetal blood samples are collected and a quantitative spectrofluorometric assay is used to determine fetal blood phenylalanine levels. Other pregnancy complications thought to be a part of the human maternal phenylketonuria syndrome are increased rates of spontaneous abortion and stillbirth. Therefore, these birth defects will also be scored in the mouse model, as well as the point in gestation at which they occur. Further, all fetuses will be analyzed for both genotype and sex in order to determine if any subset of animals is more severely affected, experience a higher incidence of birth defects, or is more susceptible to lethality. The dose response will be evaluated for each individual birth defect as a function of maternal blood phenylalanine level. Continuous variables (i.e., BW, CRL, and HC) will be assessed using regression analysis, with the goal of ultimately accepting or rejecting linearity of the dose response curve. Discrete variables (i.e., spontaneous abortion and stillbirth) will be assessed through chi-square analysis.

**Prevention of Maternal PKU: What is an adequate phenylalanine level for adolescent females and other women of childbearing age?** *G. Pridjian<sup>1,2,3</sup>, A. Cunningham<sup>1</sup>*. 1) Hayward Genetics Center; 2) Department of Pediatrics; 3) Division of Maternal and Fetal Medicine, Department of Ob/Gyn, Tulane University School of Medicine, New Orleans, LA.

Maternal PKU continues to be a devastating disease. Conception and pregnancy in the metabolic milieu of uncontrolled PKU leads to microcephaly, developmental delay/retardation, congenital anomalies (in particular congenital heart disease), and growth delay. Maternal PKU embryopathy can be prevented by maintaining maternal phenylalanine levels in a normal range (2 to 6 mg/dL). To assure normal levels of phenylalanine in the first trimester, excellent preconception metabolic control is advocated. However, 40 to 65% of pregnancies are unplanned, often discovered late in the first trimester. Statements of the NIH Consensus Development Conference include maintenance of phenylalanine values between 2 and 15 mg/dL in PKU individuals after 12 years of age, with individual consideration for gender and childbearing status. In the last 6 years alone 8 pregnancies of 4 mothers with PKU were followed in the Hayward Genetics Center. All mothers were consistently instructed regarding the teratogenesis of uncontrolled plasma phenylalanine and encouraged to plan pregnancy with prepregnancy control. Of the 8 pregnancies, 7 were unplanned. The mean gestational age at the time of pregnancy diagnosis was 9.3 menstrual weeks (range 6.5 to 16.0). The mean phenylalanine level at the time of diagnosis was 14.4 mg/dL (range 9.2 to 25.9). Of the 8 pregnancies, three have signs of maternal PKU embryopathy. Their initial values after pregnancy was discovered were 25.9, 18.7, and 16.6 mg/dL. However, all had some dietary modification prior to the initial level. Two pregnancies with no sign of maternal PKU are still ongoing. While 2 to 15 mg/dL may be an acceptable range for male adolescents with PKU, it may not be for females. While the data in this report are small in number, the outcomes are convincing to suggest female adolescents with PKU, and in general, all females with PKU of childbearing age should maintain phenylalanine levels closer to the normal range (2 to 6 mg/dL) as pregnancy is often not a planned phenomenon.

**Impact of apo E genotype on familial combined hyperlipemia (FCHL) expression.** *O. Guardamagna<sup>1</sup>, C. Bondone<sup>1</sup>, C. Sacchetti<sup>1</sup>, A. Gomez<sup>3</sup>, G. Mercadante<sup>2</sup>, E. Cocco<sup>3</sup>, C. Allora<sup>1</sup>, M. Bo<sup>2</sup>, R. Bonardi<sup>4</sup>, S. Bosso<sup>3</sup>, G. Restagno<sup>3</sup>.* 1) Pediatria, University of Torino; 2) Medicina Interna, University of Torino; 3) Molecular Genetics Service, AOOIRM-S.Anna, Torino; 4) Malattie dell'Apparato Digerente e Nutrizione, University of Torino, Italy.

The apo E gene represents a major determinant in lipoprotein metabolism. The e2, e3 and e4 alleles, which originate six different apolipoproteins, are differently related to total cholesterol (TC), triglycerides (Tg), and apo B levels. Furthermore the relationship between the familial combined hyperlipemia (FCHL, a complex inherited disorder of lipoprotein carrying apo B) and apo E haplotypes is poorly questioned. Aim of the study is to examine in the Italian FCHL kindreds the correlation between common haplotypes E2/E3, E3/E3, and E3/E4 and lipoprotein profiles. Methods: 35 affected children, 63 adults patients and 6 healthy sibs from 27 FCHL kindreds were submitted to biochemical analysis and DNA collection. The diagnosis was based on TC and/or Tg exceeding the 90<sup>o</sup> percentile, on at least a first degree related affected patient and on kindreds phenotype variability. The apo E isoforms were detected by DNA RFLPs after cleavage with CFoI enzyme. Statistical evaluation was based on ANOVA one-way method. Results: Alleles frequencies in FCHL and control kindreds were not significant. TC resulted 140, 224, 272 mg%, LDL-C 70, 149, 196 mg %, apo B 74, 109, 141 mg% and Tg 182, 89 and 142 mg% in E2/E3, E3/E3 and E3/E4 respectively. A statistically significant result (p<0.001 and p<0.05) was obtained on TC, LDL-C and apo B respectively on adults and children. Conclusions: TC, LDL-C and apo B higher levels were observed in subjects carrying the E3/E4, followed by subjects with E3/E3 and E2/E3 haplotypes. In contrast the higher Tg levels are observed in the E2/E3 haplotype. These results confirm a correlation between apo E alleles and the biochemical expression of FCHL phenotype. Moreover the atherogenic phenotype related to e4 allele is here evidenced since pediatric age, while the involvement of e2 is still doubtful.

**Detection of Hartnup's Disorder in an Alkaptonuria Sibship.** *I. Bernardini, W. Introne, R. Kleta, D.L. Fitzpatrick, W.A. Gahl.* HDB, NICHD, NIH, Bethesda, MD.

Our investigations into alkaptonuria include collecting histories and assessing routine physiological parameters of the patients enrolled. These data will yield a composite natural history of this rare disease, and provide us with a baseline against which to evaluate the effects of NTBC therapy on homogentisic acid excretion, renal glomerular function, and plasma and urine amino acid patterns. NTBC, currently used to treat type I tyrosinemia, inhibits the production of homogentisic acid, which accumulates in alkaptonuria and destroys joints and other cartilage-containing tissues. In the 44 alkaptonuria patients assessed so far, we have found normal renal glomerular function except for one patient with end-stage renal disease due to insulin-dependent diabetes mellitus. Plasma and urine amino acids have been within normal limits, except for one family in which two of the three sibs with alkaptonuria also had plasma and urine amino acid patterns consistent with Hartnup's disorder. Remarkably, all 3 sibs also carried a diagnosis of documented sucrase-isomaltase deficiency. The theoretical probability of finding these three conditions in a single individual by chance alone would be approximately one in 4 trillion. However, in this case the family history revealed a coefficient of inbreeding of 1/8 (Garnica, 1981). Hartnup's disorder is an abnormality of renal tubular and gastrointestinal mucosal transport of neutral amino acids at the plasma membrane level. The main symptoms result from niacin deficiency, since this vitamin/cofactor is synthesized from tryptophan, one of the amino acids that fails to be absorbed properly in the gastrointestinal tract. In our two affected siblings, neutral amino acids were low in plasma and high in urine, resulting in elevated fractional excretions. The patients had histories of gastrointestinal and neuropsychiatric symptoms consistent with symptomatic Hartnup's disorder. After confirmation of this abnormality, a trial of niacin supplementation will be attempted. This case emphasizes the possibility that multiple rare disorders can occur in a single individual, especially when consanguinity is involved.

**Misconceptions and Concerns Regarding the Use of Tandem Mass Spectrometry in Newborn Screening.** *D.H. Chace, S.M. Beatty, E.W. Naylor.* Neo Gen Screening, Bridgeville, PA.

Tandem Mass Spectrometric (MS/MS) analysis of dried blood specimens for acylcarnitines and amino acids is clearly one of the most significant advances in newborn screening since Robert Guthrie introduced BIA's in the early 60s to detect PKU. As quoted by Harvey Levy in a Clinical Chemistry editorial, MS/MS analysis represents a sea-change in how newborn screening is approached. No longer is one method used to analyze one metabolite to detect one disorder. The new approach is to use one method to analyze multiple metabolites to detect multiple disorders. With future applications of molecular diagnostics, this concept will be clearly established as the norm. As the use of MS/MS is rapidly growing throughout the US and other nations. What began as a research/clinical screening tool for an analysis of a few samples averaging 10-20 infants per day in the early 1990s has grown to 1000+ samples per week in one laboratory. Today, more than 15% of the infants born in the US now have at least one metabolite measured using MS/MS. Clearly, the benefit of MS/MS screening is being born out with statistics now being obtained. However, there still remains much confusion and misinformation regarding the appropriate use of MS/MS as well as the research that is still required to validate and educate physicians about this extraordinarily comprehensive, complex tool. Issues with regard to the diseases that may be detectable by MS/MS, the differences between screening programs using this technology, the numerous alterations of a method to suite individual laboratory needs, the early stages of implementation of QA, challenges of interpretation, disease treatability and challenges in implementation will be discussed. MS/MS is clearly an important system for enhanced newborn screening and raises issues that will have to be discussed and solved as more complex comprehensive diagnostic methods are developed. MS/MS provides us with an early opportunity to consider new approaches, programs and issues that must be solved such as QA, informed consent, education, follow-up and treatment of more than 100 diseases affecting newborns that may be analyzed in some laboratories.

**Neutral lipid storage disease with mild ichthyosis, hepatopathy, developmental delay and skeletal anomalies (Chanarin-Dorfman disease).** *E. Morava*<sup>1</sup>, *T. Decsi*<sup>2</sup>, *L. Szonyi*<sup>3</sup>. 1) Department of Clinical Genetics, Pecs University, Medical Faculty, Pecs, Hungary; 2) Department of Pediatrics, Pecs University, Pecs, Hungary; 3) Department of Pediatrics, Semmelweis University, Budapest, Hungary.

Neutral lipid storage in liver, muscle and other tissues is a rare metabolic condition. It may be present due to lysosomal acid lipase deficiency which is rapidly fatal in Wolmann's disease but may also be compatible with long term survival in Chanarin-Dorfman disease. Characteristic clinical features are developmental delay with muscle hypotonia, hepatomegaly with increased liver enzyme activities and ichthyosis. Lipid laden leukocytes (Jordan's anomaly) are commonly observed. We report on a male infant with severe muscle hypotonia and liver steatosis in whom mild ichthyosis rose the suspicion of Chanarin-Dorfman disease. The diagnosis was corroborated by detecting elevated hexacosanoic acid (C26:0) values in plasma lipids. We also report multiple skeletal anomalies which represent a hitherto unreported finding in this syndrome.



**Early retinal and MRI changes in a neonate with homocystinuria and methylmalonic aciduria(*cb1C*).** P. Jayakar<sup>1</sup>, E. Vasconsellos<sup>1</sup>, D.S. Rosenblatt<sup>2</sup>. 1) Miami Children's Hospital, Miami, FL; 2) McGill University, Montreal, Canada.

AA, a girl born of non-consanguineous Mexican parents was doing well on breast feeds till the age 3 weeks, when she presented with respiratory infection, progressive lethargy, poor feeding and bilateral retinal hemorrhages. The brain CT and the bone survey were normal. At age 1 month she had low plasma methionine(4umoles/L), elevated free plasma homocystine(46 umoles/L) and normal serum vitamin B12. Urine organic acids showed elevated methylmalonic acid (MMA) (177.4mmol/molcr) and methylcitric acid. The uptakes of C14-propionate and C14-methyltetrahydrofolate by cultured fibroblasts were low with a good response to hydroxycobalamin in culture medium; complementation studies confirmed the (*cb1C*)defect. On starting hydroxycobalamin(1mg/day)and carnitine, plasma free homocystine decreased (9umoles/L), plasma methionine (14umoles/L)normalized and urine MMA decreased(10.9mmol/molcr) within 2 to 5 days. A brain MRI at 1 month showed lateral and third ventriculomegaly, cerebral atrophy, spongiform changes and increased signal in the periventricular white matter extending to the subcortical U fibers, normal basal ganglia and cerebellum. Ophthalmic exam showed subtle pigmentary macular changes, several small faded blot hemorrhages in the mid periphery along the veins and temporal arcade, 2 tiny linear dark and fresh hemorrhages bilaterally in the macula with absent macular reflex. At age 3 months, ophthalmic exam showed optic atrophy, no new hemorrhages and MRI showed progressive ventriculomegaly, prominent basal cisterns, increased signal and thinning of periventricular white matter in the right hemisphere, mild cerebellar atrophy, with normal basal ganglia. At 5 months, she is globally delayed with no metabolic crisis, normal plasma methionine and plasma free homocystine but mildly elevated urine MMA (15.2mmol/molcr). The neonatal presentation of lethargy and retinal hemorrhages is unusual for (*cb1C*) and needs to be differentiated from non-accidental trauma. The diagnostic difficulties are further compounded by the early white matter spongiform changes typically seen in other neurodegenerative disorders; these progressed despite early treatment.

**Biochemical and molecular analysis of 12 patients with thiamine-responsive pyruvate dehydrogenase complex deficiency.** *E. Naito, M. Ito, I. Yokota, T. Saijo, Y. Ogawa, K. Shinahara, S. Yamamoto, Y. Kuroda.* Department of Pediatrics, School of Medicine, University of Tokushima, Tokushima, Japan.

We report biochemical and molecular analysis of 12 patients with thiamine-responsive pyruvate dehydrogenase complex (PDHC) deficiency. PDHC activity was assayed using two different concentrations of thiamine pyrophosphate (TPP) in cultured lymphoblastoid cells or cultured fibroblasts from 12 patients. These 12 patients displayed very low PDHC activity in the presence of a low ( $1 \times 10^{-4}$  mM) TPP concentration. These PDHC activities increased at a high (0.4 mM) TPP concentration. Especially, PDHC activity of six patients increased within normal range. Therefore, PDHC deficiency in 12 patients was due to a decreased affinity of PDHC for TPP. Treatment with a high dose of thiamine resulted in a reduction in lactate and clinical improvement in these patients, suggesting that these 12 patients have a thiamine-responsive PDHC deficiency and a high dose of thiamine is very effective for patients with this type of PDHC deficiency. The DNA sequence of these patients' X-linked E1a subunit revealed a point mutation in all 12 patients, including 6 patients in exon 3, one patient in exon 4, two patients in exon 7, and three patients in exon 8. Five mutations (V71A, R88C, C101F, L216F, and L260Q) of them were novel. Two mutations (L216F and R263G) were found in the genomic DNA of two mothers, and the PDHC activity of these two mothers was decreased in the presence of a low TPP concentration. Half of 12 patients with thiamine-responsive PDHC deficiency were caused by mutations in exon 3 of E1a subunit gene. Therefore, exon 3 in the E1a subunit gene appears to be important in thiamine-responsive PDHC deficiency.

**Biochemical, behavioral and pathologic analysis of an animal model of glutaric acidemia type I.** *D.M. Koeller<sup>1</sup>, L.S. Crnic<sup>2</sup>, M. Wootner<sup>2</sup>, B.K. DeMasters<sup>3</sup>, J. Stephens<sup>3</sup>, E.L. Hunt<sup>3</sup>, S.I. Goodman<sup>2</sup>.* 1) Depts Pediatrics and Molecular & Medical Genetics, Oregon Health Sci Univ, Portland, OR; 2) Dept Pediatrics, Univ of Colorado Health Sci Ctr, Denver, CO; 3) Dept of Pathology, Univ of Colorado Health Sci Ctr, Denver, CO.

Glutaric acidemia type I (GA-I) is an autosomal recessive disorder of organic acid metabolism resulting from a deficiency of glutaryl-CoA dehydrogenase (GCDH). Patients accumulate glutaric acid (GA) and 3-OH glutaric acid (3-OHGA) in their blood, urine and CSF. Clinically, GA-I is characterized by macrocephaly, progressive dystonia and dyskinesia. Degeneration of the caudate and putamen of the basal ganglia, widening of the Sylvian fissures, fronto-temporal atrophy and severe spongiform change in the white matter are also commonly observed. To study the pathophysiologic basis for the neurologic damage seen in GA-I we made a mouse model via targeted deletion of the *Gcdh* gene in embryonic stem cells. *Gcdh*<sup>-/-</sup> mice accumulate both GA and 3-OHGA at levels similar to those seen in GA-I patients. The affected mice have a mild motor deficit but do not develop the progressive dystonia seen in human patients. Subjecting the *Gcdh*<sup>-/-</sup> mice to a metabolic stress, which often precipitates symptoms in patients, failed to have any neurologic effect on the mice. Pathologically, the *Gcdh*<sup>-/-</sup> mice have a diffuse spongiform myelinopathy similar to that seen in GA-I patients. However, unlike in human patients, there is no evidence of neuron loss or astrogliosis in the striatum.

**Study of two phosphate transporters of the Anion/Cation Symporter family in GSD1c.** *D. Melis<sup>1</sup>, G.M.S. Mancini<sup>2</sup>, A. Havelaar<sup>2</sup>, A. Benedetti<sup>3</sup>, G.P.A. Smit<sup>4</sup>, F.W. Verheijen<sup>2</sup>.* 1) Dept of Pediatric, Federico II University, Naples, Italy; 2) Department of Clinical Genetics, Erasmus University, Rotterdam; 3) Department of Pathology, University of Siena; 4) University Hospital, Groningen.

The Anion/Cation symporter (ACS) family is a relatively large group of phylogenetically conserved transporter genes. Recently two phosphate transporters of the ACS family, NPT3 and NPT4, have been identified; northern blot analysis and RT-PCR revealed that they are expressed in liver, kidney and intestine. Aim of the present study was to analyze the intracellular localization and the genomic structure of human NPT3 and NPT4. Human NPT4 was expressed from the vector pcDNA3.1/GS (Invitrogen). The expression in COS-1 cells and the immunofluorescence study showed an endoplasmic reticulum (ER) localization. The high homology of NPT3 and NPT4 and the analysis of the putative aminoacid sequence points to an ER localization also for NPT3. The genomic structure showed 11 exons in NPT3 and 10 exons in NPT4. The deficiency of a microsomal phosphate transporter in liver, kidney and intestine has been suggested as the biochemical basis of glycogen storage disease type Ic (GSDIc), but mutations in phosphate transporters have not been found yet. We sequenced NPT3 and NPT4 in DNA from six patients affected by GSDIc in whom mutations in both glucose-6-phosphatase (G6Pase) and G6PT genes had not been found. One patient was heterozygous for a G to A transition at nucleotide 920 determining the glycine 307 be replaced by an arginine, in NPT4. In NPT3, a different patient was heterozygous for a C to T transition at nucleotide 1527 determining the serine 509 be replaced by a leucine and two alterations were also detected in the intronic regions in three patients. These alterations were not detected in 10 controls. These results are not conclusive to confirm or exclude the involvement of NPT3 and NPT4 in GSDIc. However, our studies suggest a role of NPT3 and NPT4 in the regulation of phosphate availability in the ER. Presumably, high concentration of phosphate inhibits the G6Pase. The existence of GSDIc patients with mutations in NPT3 and NPT4 suggests a regulatory function of these transporters on G6Pase system.

**Determination of the complete DNA sequence of the human phenylalanine hydroxylase gene.** *D.S. Konecki, U. Lichter-Konecki.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

Classical phenylketonuria (PKU), an inborn error of amino acid metabolism, is inherited in an autosomal recessive manner and usually results from a deficiency of the enzyme phenylalanine hydroxylase (PAH). A cDNA encoding the human PAH enzyme was isolated and characterized in the laboratory of S.L.C. Woo at Baylor College of Medicine in 1983. The PAH gene structure was also determined in the Woo laboratory and published in 1986. Since that time more than 300 mutations in the PAH gene have been documented to result in PKU and a number of studies have been published increasing the understanding of the regulation of this gene. An obvious absence from this research landscape has been the complete sequence of the human PAH gene. We first began generating and collecting PAH gene sequences in 1984. During the last two years we have been involved in a dedicated effort to determine the complete sequence of the human PAH gene through the use of random and directed sequencing approaches applied to isolated human lambda and BAC genomic recombinants containing the gene. More than 1000 sequence reads from subcloned genomic fragments and sequences directed by 426 PAH gene-specific primers are included in the ~79kbp PAH gene sequence. The entire length of the finished genomic sequence encompassing the human PAH gene is ~171kbp in length and extends ~27kbp upstream of the transcription initiation site in the first exon and terminates ~64.5kbp downstream of the poly(A) addition site located in the last exon of the gene (exon 13). Also included are PAH genomic sequences obtained from the NCBI sequence databases. It is anticipated that the completed sequence of the human PAH gene will be of value to the PAH/PKU research community and to investigators studying genetic diversity through variations in the human PAH gene.

**Mitochondrial Trifunctional Protein Defects and Sudden Infant Death Syndrome.** *Z. Yang, P. Lantz, Y. Zhao, J.A. Ibdah.* Wake Forest University School of Medicine.

Background: Infants with fatty acid oxidation (FAO) disorders may die suddenly and unexpectedly, and, if appropriate investigations are not conducted, may meet the criteria for sudden infant death syndrome (SIDS). Biochemical studies have suggested that »5% of all SIDS cases have FAO defects. The objective of this study is to characterize the association between defects in the mitochondrial trifunctional protein (MTP) and SIDS in families with documented MTP mutations and in SIDS cases with fatty infiltration of the liver. MTP is the enzyme complex that catalyzes the last 3 steps of long chain FAO. Methods: We analyzed the pediatric history and genotypes in 34 families with documented MTP mutations. We also reviewed the histopathological changes in 120 SIDS cases certified by the regional Medical Examiner. Cases with fatty infiltration of the liver were screened for mutations in MTP. Results: In families with MTP defects, 13/34 (38%) of the affected children died. Four deaths were sudden, and initially unexplained at 4-8 months of age. Autopsy revealed diffuse micro- and macro-vesicular hepatic steatosis. All were homozygous for the common G1528C (E474Q) mutation. In five families there was a history of sudden and unexplained death in a sibling of an unknown genotype. Our retrospective analysis in SIDS cases revealed diffuse micro- and macro-vesicular hepatic steatosis in 13/120 (»11%) cases. Molecular analysis identified 1/13 (»8%) with compound heterozygosity for MTP defects. The first mutation was the common G1528C mutation. The second mutation was a 1-bp deletion at position 1967 (1967T) in exon 18, which causes a stop codon and a premature termination. Additional screening for the common G985A mutation in medium chain acyl-CoA dehydrogenase (MCAD) revealed homozygosity in another case and heterozygosity in two. Conclusions: MTP defects cause sudden unexpected death and account for a significant proportion of SIDS cases with fatty infiltration of the liver. We recommend screening for FAO defects in SIDS cases with fatty infiltration of the liver. (Supported by a grant #6-FY99-376 from March of Dimes).

**Molecular basis of autosomal recessive desmosterolosis.** R. Waterham<sup>1,2</sup>, J. Koster<sup>1</sup>, G.J. Romeijn<sup>1</sup>, P. Vreken<sup>1</sup>, R.C.M. Hennekam<sup>2</sup>, H.C. Andersson<sup>3</sup>, D. FitzPatrick<sup>4</sup>, R.I. Kelley<sup>5</sup>, R.J.A. Wanders<sup>1,2</sup>. 1) Lab Gen Metabolic Dis (FO-224), Acad Med Center/Univ Amsterdam, Amsterdam, Netherlands; 2) Dept. of Pediatrics, Acad Med Center, Amsterdam, Netherlands; 3) Tulane University School of Medicine, New Orleans, USA; 4) MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK; 5) Kennedy Krieger Institute, Baltimore, USA.

Recent years, several inherited disorders have been linked to enzyme defects in the isoprenoid (cholesterol) biosynthetic pathway by the finding of abnormally increased levels of intermediate metabolites in patients followed by the demonstration of disease-causing mutations in genes encoding the implicated enzymes [refs 1-4 for reviews]. Most currently known enzyme defects in the pathway specifically affect cholesterol biosynthesis and lead to multiple congenital anomalies, and/or skeletal and skin abnormalities underlining cholesterol's pivotal role in embryogenesis/morphogenesis. We now report the molecular basis of autosomal recessive desmosterolosis (MIM 602398), a severe multiple congenital anomaly syndrome. Affected patients have elevated levels of desmosterol in tissues, plasma and cultured cells, indicating a deficiency of 3 $\beta$ -hydroxysterol D<sup>24</sup>-reductase (DHCR24), the enzyme catalysing the reduction of the C24-C25 (D<sup>24</sup>) double bond of cholesterol precursors including desmosterol. By similarity searching using a candidate gene of the plant *Arabidopsis thaliana*, we identified the gene encoding human DHCR24 (*DHCR24* at chr. 1) as demonstrated by expression in yeast. Sequence analysis of *DHCR24* in genomic DNA of two patients with desmosterolosis identified four different missense mutations confirmed to be disease-causing by expression in yeast and revealing a clear genotype-phenotype correlation. Our results demonstrate that mutations in *DHCR24* cause desmosterolosis. {Supported by a fellowship of the Royal Netherlands Academy of Sciences to HRW}

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**Biochemical and molecular analysis of a patient with Congenital Disorder of Glycosylation type Ia.** *T.C. Wood<sup>1</sup>, D. Flannery<sup>2</sup>, J. Kottra<sup>3</sup>, J. Longshore<sup>1</sup>, H. Taylor<sup>1</sup>.* 1) Greenwood Genetic Ctr, Greenwood, SC; 2) Department of Pediatrics, Medical College of Georgia, Augusta, GA; 3) Eisenhower Army Hospital, Augusta, GA.

Congenital disorder of glycosylation (CDG) comprise a group of inherited disorders caused by defects in N-glycosylation. The most common type, CDG Ia, results from a deficiency of phosphomannomutase (PMM) that normally converts mannose-6-phosphate to mannose-1-phosphate. Clinically CDG Ia patients can present with strabismus, psychomotor retardation, retinitis pigmentosa, dysmorphic features, inverted nipples, and abnormal fat pads. Screening for CDG is usually performed with serum transferrin and diagnosis of CDG Ia is made by measuring PMM activity. The phosphomannomutase gene (PMM2) has been isolated and mutational analysis has been performed on a number of patients. We report on a patient referred because of growth failure, strabismus, muscle spasms, inverted nipples, and an accumulation of fat above the hips. Capillary electrophoresis of the patients serum showed a change in the transferrin sialoform pattern, consistent with CDG. Sequencing analysis of the PMM2 gene identified three changes in the coding region: G42R, I153T, and E197A. The latter two changes have been identified as causative mutations in other CDG Ia patients, however G42R has not been previously reported. The glycine involved in the G42R mutation is conserved between the human and *Drosophila melanogaster* PMM proteins, and this change has not been noted in the other CDG Ia patients in which the PMM2 gene has been sequenced. Studies are currently underway in an attempt to determine if this change is a polymorphism. Analysis of the patients parents found the father to carry the I153T allele while the mother carries both the G42R and E197A alleles. Because both the G42R and E197A alleles were passed from the mother, and because she is healthy, we suggest that they are located on the same chromosome, in cis position, in the proband.



**Kelley-Seegmiller syndrome as a novel cause of gout in a female patient.** *I. Sebesta<sup>1,2</sup>, L. Dvorakova<sup>1</sup>, M. Kumsta<sup>2</sup>, B. Stiburkova<sup>1</sup>, S. Kmoch<sup>1</sup>, J.A. Duley<sup>3</sup>, H.A. Simmonds<sup>3</sup>.* 1) Inst.Inher.Metab.Dis; 2) Inst.Clin.Biochem., 1st Fac.Med., Charles Univ., Prague, Czech Republic; 3) Purine Research Unit, Guy's Hospital, London, UK.

Partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) described as Kelley-Seegmiller (KS) syndrome is an X-linked recessive genetic defect associated with overproduction of uric acid. Complete HPRT deficiency (Lesch-Nyhan syndrome) results in severe neurological symptoms and urate overproduction. KS syndrome generally presents later with gout and/or urolithiasis and has been reported exclusively in males. Female carriers have somatic cell mosaicism of HPRT activity, and are healthy. We have found a girl with partial HPRT deficiency. She presented at the age of 9 years with gouty arthritis of big toe and hyperuricaemia. No evidence of neurological involvement was found. At the age of 16 years she was referred for purine metabolic investigation. Activity of phosphoribosylpyrophosphate synthetase in erythrocytes was normal. Absence of HPRT activity, together with raised activity of adenine phosphoribosyltransferase in lysed erythrocytes and raised concentration of nicotinamide adenine dinucleotide in erythrocytes confirmed partial HPRT deficiency. The loss of HPRT activity was found in the girl's father, who has suffered renal colic and gout since the age of 18 years. The father's brother also has the same clinical and biochemical findings. Molecular genetic analysis revealed a missense mutation in the patient and her father. It is a T→C transition at position 158 of the cDNA that changes codon for valine to alanine (V53A). This mutation has also been described in a male patient with partial HPRT activity (K.Sege-Peterson et al,1992, Hum.Mol.Gen. 1:427-32). X chromosome inactivation studies are in progress. This unusual and first description of partial HPRT deficiency in a female enlarges the differential diagnosis of hyperuricaemia and/or gout in females and underlines the need to perform purine metabolic investigations in such findings. Gout in the young should always be investigated.(Supported by grants:EC BMH4-CT98-3079;MSM - 111100005, Czech ME and IGA MH NE 6559-3-01).

**Pediatric reference ranges for delta-aminolevulinic acid(ALA), porphobilinogen(PBG), and fractionated porphyrins in urine.** *D. Salazar, L. Guerrero, J.E.S. Lee.* Dept Biochemical Genetics, Quest Diagnostics Nichols Inst, San Juan Capist, CA.

**Introduction:** Most genetic forms of porphyria are inherited as autosomal dominant, leading to a heterozygous state in the majority of patients. Individuals most often manifest at or after puberty; thus, porphyrias are generally thought of as adult disorders. Since individuals do sometimes present during childhood, we developed pediatric reference ranges based on random urine specimens rather than the more-difficult-to-collect 24-hour urine specimens.

**Methods:** Random urine samples were collected from 95 apparently healthy, ambulatory children: 47 females, 48 males, ages 1-17.9 years. Children with chronic illness or an acute illness of longer than 2 weeks duration were excluded from the study. Samples were light protected and frozen at -70°C prior to analysis. ALA and PBG were analyzed using kits from Bio-Rad Laboratories (Hercules, CA). Porphyrins were fractionated by HPLC and detected by fluorescence.

**Results:** ALA, PBG and fractionated porphyrin levels were independent of sex, but not age. The ALA reference range is 2.3-6.2 mg/g Cr for 1.0-8.9 years and 1.5-5.3 mg/g for 9.0-17.9 years. The PBG reference range is 0.9-2.8 mg/g Cr for 1.0-8.9 years and 0.5-2.0 mg/g Cr for 9.0-17.9 years. The fractionated porphyrin reference ranges are 4.3-16.2 mg/g Cr for uroporphyrin, <4.6 mg/g for heptaporphyrin, not detected for hexaporphyrin, <3.2 mg/g for pentaporphyrin, 10.1-254.7 mg/g for coproporphyrin and 17.0-269.7 mg/g for total porphyrins at 1.0-10.9 years. The reference ranges for 11.0-17.9 years are 4.6-18.9 mg/g Cr for uroporphyrin, <4.6 mg/g for heptaporphyrin, not detected for hexaporphyrin, <3.0 mg/g for pentaporphyrin, 11.8-107.2 mg/g for coproporphyrin, and 16.4-121.5 mg/g for total porphyrins.

**Conclusion:** Concentrations in the pediatric population are generally higher than in the adult population. Thus, these reference ranges will be useful for diagnosing porphyria in the pediatric population.

**A novel common mutation in OCTN2, carnitine transporter gene, in Southern Chinese patients with primary carnitine deficiency.** *N.L.S. Tang<sup>1</sup>, W.L. Hwu<sup>2</sup>, R.T. Chan<sup>3</sup>, S.L.M. Fung<sup>1</sup>, L.K. Law<sup>1</sup>.* 1) Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong; 2) Department of Medical Genetics, National Taiwan University Hospital, Taiwan; 3) Department of Pediatrics and Neonatology, Centro Hospitalar Conde S. Januario, Macau.

Mutations in OCTN2, encoding for the plasma membrane carnitine transporter cause primary carnitine deficiency. After our first report of mutations in Chinese, we collected other Chinese families with the disease for mutation analysis. Two families were recruited from Taiwan and one family was from Macau. All patients are Southern Chinese, originating from Canton Province. The parents of these families were non-consanguineous and unrelated. Mutation analysis was performed by PCR of all ten exons and PCR products were directly sequenced. Two novel truncating mutations were detected. A single base mutation (C to T) was found at cDNA position 981 of codon 254 (CGA->TGA). Another mutation was at codon 387 (TAT->TAG). Two probands, one each from Taiwan and Macau, were homozygote for R254X. The other proband from Taiwan carried R254X and Y387X. Analysis of samples from parents or siblings indicated that the mutations were inherited but not sporadic mutation. Two heterozygote carriers of R254X were also identified among 250 control subjects, while none was detected from Y387X. It suggests that the population carrier rate for R254X was around 1/125. Haplotype of R254X was determined with three intragenic polymorphisms, including silent polymorphisms of codon 95 and 269, and an intron polymorphism, ivs4+13C/T. All five chromosomes carrying R254X showed the same haplotype on these markers. Analysis of population allele frequencies of these markers indicates the chance of these five chromosomes with R254X arising independently was less than 0.05. This study revealed novel mutations in OCTN2 found in the Southern Chinese population. The R254X may be a founder mutation and its carrier frequency in the general population may be about 1 in 125. The predicted disease prevalence due to R254X alone would be 1 in 60,000 suggesting primary carnitine deficiency is a relatively common inherited metabolic disease in the locality.

**A novel defect of N-glycan synthesis.** *F. Scaglia<sup>1</sup>, J.L. Northrop<sup>1</sup>, C.N. Ou<sup>2</sup>, D.G. Patel<sup>3</sup>, M.A. Gilger<sup>3</sup>, S.J. Karpen<sup>3</sup>, V. Westphal<sup>4</sup>, H.H. Freeze<sup>4</sup>.* 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept Pathology, Baylor Col Medicine, Houston, TX; 3) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 4) The Burnham Institute, La Jolla, CA.

The congenital disorders of glycosylation (CDGs) are autosomal recessive disorders, with a spectrum of neurologic and multisystemic manifestations caused by defective N-glycosylation. Different types of CDGs can be recognized by altered isoelectric focusing patterns of serum transferrin. We report a 10 month-old male infant with protein losing enteropathy, liver disease, coagulopathy and developmental delay. He initially came to medical attention at 6 weeks of age with the symptoms of diarrhea and hypoalbuminemia. During his hospital course he developed protein losing enteropathy, hypotonia and liver disease. His condition was complicated by anasarca, seizures, and severe coagulopathy. In addition, he developed hypothyroidism, cataracts and decreased bone mineralization that led to fractures. Due to onset of seizure activity, a brain MRI was obtained and it revealed brain volume loss and a diffuse leukoencephalopathy with no cerebellar hypoplasia. Genetics was consulted and a congenital disorder of glycosylation was considered. A diagnosis of CDG type I was made on an abnormal serum transferrin isoelectric focusing pattern. Normal phosphomannomutase and phosphomannose isomerase activities in leukocytes ruled out CDGs type Ia and type Ib. CDG type Ic was excluded based on the sequencing of the ALG6 gene. CDGs type Id and type Ie were ruled out based on biochemical analyses and sequencing of the DPM1 gene respectively. Evaluation for the cause of CDG type If is currently underway with cDNA sequencing analysis of MPDU1 and *in vitro* studies of the synthesis of N-linked glycans in fibroblasts to identify the defective enzyme. It is important to increase awareness of these disorders since many patients with glycosylation defects probably remain undiagnosed. This case report shows that the association of protein losing enteropathy, coagulopathy and liver dysfunction is not necessarily associated only with CDG type Ib and that the spectrum of this type of disorders continues to expand.

**A NOVEL MUTATION OF THE ALPHA-GALACTOSIDASE A GENE IN A FAMILY WITH A JUVENILE-ONSET FORM OF FABRY DISEASE.** *Y.S. Shin*<sup>1</sup>, *T. Podskarbi*<sup>2</sup>. 1) Pediatrics, University of Munich, 80337 Munich, Germany; 2) Molecular Genetics and Metabolism Laboratory, Munich, Germany.

Fabry disease is an X-linked metabolic disease which is caused by deficiency of the lysosomal alpha-galactosidase A. The disease results in the progressive globotriaosylceramide (GL-3) accumulation primarily in vascular endothelium. We report here a German male with typical changes of Fabry disease mainly in the skin and eyes. Even though leading cardio-, vaso-, reno-, oculo- and cutaneous symptoms were there since early childhood, the diagnosis of Fabry disease has been delayed until his first visit to the hospital at the age of 22 years. The alpha-galactosidase A activity in leukocytes was significantly decreased (0.02 nmol/min/mg protein; mother: 0.35; normal range: 0.4-1.2). The sequence analysis of the alpha-galactosidase A gene revealed the hemizygous transition in the position c.1038T>C yielding the amino acid exchange of cysteine to arginine in the codon 347 (C347R) in the patient as well as in the mother. This missense mutation possibly encodes the slight residual activity found in the patient. The molecular genetic analysis provides the definitive diagnosis of female carriers, since some heterozygotes are symptomatic and the enzyme activity values of leukocytes are not always clearly distinguishable from those of normal controls.

**Missense model of MPS VII produced by targeted mutagenesis: Genotype/phenotype correlation and immune tolerance.** *S. Tomatsu*<sup>1,2</sup>, *K. Orii*<sup>1,2</sup>, *J.H. Grubb*<sup>1</sup>, *E.M. Snella*<sup>1</sup>, *C. Vogler*<sup>3</sup>, *W.S. Sly*<sup>1</sup>. 1) Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, St. Louis, MO; 2) Department of Pediatrics, Gifu University, Gifu, Japan; 3) Department of Pathology, Saint Louis University School of Medicine, St. Louis, MO.

MPS VII is an autosomal recessive disease caused by b-glucuronidase deficiency, leading to a wide range of clinical phenotypes from the most severe hydrops fetalis type to mild types without mental retardation. To study missense mutant MPS VII models with phenotypes of varying severity, we produced L176F (the commonest human mutation) and E540A and E540Q (active site nucleophile replacement) mouse models. The E540A mouse has no GUSB activity and displays a severe phenotype like the original MPS VII mouse. Both L176F and E540Q show levels of residual activity below 0.5% and milder phenotypes. All three revealed progressive vacuolar storage in many tissues, with rate and extent of accumulation correlating with severity. These missense models should be useful in studying pathogenesis due to specific point mutations. Cellular and humoral immune responses have been recognized as impediments to enzyme replacement and gene therapy strategies. To solve this issue for evaluating long-term effectiveness and side effects of therapies for MPS VII, we introduced both the hGUSB E540A cDNA transgene into intron 9 and the active site E540A mutation into the endogenous GUS gene. These mice have no b-glucuronidase activity, and show the full MPS VII phenotype. However, ubiquitous expression of the inactive hGUSB produced tolerance to immune challenge with hGUSB. This provides a model to study long-term administration of hGUSB, the likely form of enzyme for human clinical trials, and for gene therapy with vectors expressing hGUSB.

**Medium-chain acyl Co-A dehydrogenase deficiency: identification and characterization of three novel mutations.**

*B-Z. Yang, M.R. Mysliwiec, J-H. Ding, C.R. Roe.* Inst Metabolic Disease, Baylor Univ Medical Ctr, Dallas, TX.

Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency is the most common inherited defect of fatty acid oxidation characterized by fasting intolerance, which may lead to hypoglycemia, coma, and sudden death. Most children present with acute illness between three and 15 months of age. A common mutation A985G has been identified among patients with MCAD deficiency, which accounts for 88.9 percent of the mutant allele. In this study, we report three novel mutations identified in three unrelated patients with MCAD deficiency. The diagnoses were based on plasma acylcarnitine profile, which showed highly elevated octanoyl carnitine, indicating MCAD deficiency. Using our Multiple Specific Fluorescent Primer Extension (MSFPE) assays to screen all known-mutations, only one A985G mutant allele was identified in each patient. To investigate the second mutant allele, all of MCAD exons from patients' DNA were amplified and sequenced. Sequence analysis revealed a novel A503G mutation in patient 1 and his mother, resulting in an Aspartic Acid to Glycine substitution at codon 158 (D158G) in one allele. Patient 2 was compound heterozygous for the common mutation A985G and a novel mutation, a single T insertion at nucleotide position 245. Patient 3 was also compound heterozygous for the A985G mutation and a novel mutation C250T, which results in a Leucine to Phenylalanine substitution at codon 84 (L84F). In addition, the frequency of the A985G mutant allele as well as other mutations in 35 new patients with MCAD deficiency are briefly discussed.

**Mutations in BCS1, a mitochondrial respiratory chain assembly gene, are responsible for complex III deficiency in patients with tubulopathy, encephalopathy and liver failure.** *P. de Lonlay<sup>1,5</sup>, I. Valnot<sup>1</sup>, A. Barrientos<sup>2</sup>, M.*

*Gorbatyuk<sup>2</sup>, A. Tzagoloff<sup>2</sup>, J.W. Taanman<sup>3</sup>, D. Chretien<sup>1</sup>, N. Kadhom<sup>1</sup>, A. Lombes<sup>4</sup>, H. Ogier de Baulny<sup>6</sup>, P. Niaudet<sup>5</sup>, A. Munnich<sup>1</sup>, P. Rustin<sup>1</sup>, A. Rotig<sup>1</sup>.* 1) INSERM U393, Hosp Necker-Enfants Malades, Paris, France; 2) Department of Biological Sciences, Columbia University, New-York, USA; 3) Royal Free and University College Medical School, Department of Clinical Neurosciences, London, UK; 4) INSERM U523, Institut de Myologie, Paris, France; 5) Departement de Pediatrie, Hosp Necker-Enfants Malades, Paris, France; 6) Service de Neuropediatrie, Hosp Robert-Debre, Paris, France.

Complex III (CIII, ubiquinol cytochrome c reductase) of the mitochondrial respiratory chain catalyzes electron transfer from succinate and NAD-linked dehydrogenases to cytochrome c. CIII is made up of 11 subunits of which all but one (cytochrome b) are encoded by nuclear DNA (nDNA). CIII deficiencies cause a variety of clinical symptoms including myopathy, cardiomyopathy and tubulopathy. Although pathogenic mutations in the mitochondrial cytochrome b gene have been occasionally described, no mutations in the nuclear DNA-encoded catalytic subunits have been hitherto reported in CIII deficiency. This suggests that most non-maternally transmitted disease-causing mutations lie in gene products affecting assembly of the complex. Various nuclear genes have been involved in assembly of yeast CIII. Up till now however only one such gene, BCS1, has been identified in the human genome. BCS1 represents therefore an obvious candidate gene in CIII deficiency. Here, we report BCS1 mutations in six patients, from four unrelated families, presenting neonatal proximal tubulopathy and hepatic involvement. Complementation studies in yeast confirmed the deleterious nature of these mutations. In addition to being the first reported nuclear gene responsible for isolated CIII deficiency, this gene is apparently a frequent cause of CIII deficiency, as one third of our complex III-deficient patients carried BCS1 mutations.



**A mutation in mitochondrial DNA encoded cytochrome c oxidase II gene in a child with Alpers-Huttenlocher - like syndrome.** *S. Finnila*<sup>1,2,5</sup>, *J. Uusimaa*<sup>2,3,5</sup>, *L. Vainionpää*<sup>3</sup>, *R. Herva*<sup>4</sup>, *H. Rantala*<sup>3</sup>, *I.E. Hassinen*<sup>2</sup>, *K.*

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Defects in mitochondrial oxidative phosphorylation are common causes of unexplained childhood encephalomyopathies, but the common mitochondrial DNA mutations are rarely found in these patients. We detected a combined defect in respiratory chain complexes I and IV in a girl with psychomotor retardation, hypotonia, fatty liver, cardiomyopathy and lactic acidosis. The disease proved fatal at the age of 6 years 8 months. Postmortem histological examination revealed a marked loss of neurons in the olivary nuclei and a spongy change in the calcarine cortex, fatty infiltration and micronodular cirrhosis of the liver and atrophic ovaries. Determination of the sequence of the entire coding region of mitochondrial DNA showed a novel heteroplasmic 7706G>A mutation in the cytochrome c oxidase subunit II. The median degree of the mutant heteroplasmy in five tissues was 90 %. The degree of the mutant heteroplasmy was 87 % in the blood of the proband, 72 % in the blood of probands asymptomatic mother and 66 % in the blood of probands asymptomatic grandmother. The 7706G>A mutation converts a hydrophobic alanine in a highly conserved transmembrane segment to hydrophilic threonine. We suggest that the 7706G>A mutation is pathogenic and leads to defective assembly of cytochrome c oxidase by disrupting a hydrophobic-hydrophobic interaction between subunits II and VIc. The clinical phenotype of this patient resembled that in Alpers-Huttenlocher disease suggesting that analysis of mitochondrial DNA may be worthwhile in patients with a progressive cerebrohepatic disease.

**Comparison studies in four different mutations located in the ATPase 6 gene.** *R. Carozzo<sup>1</sup>, O. Capuano<sup>1</sup>, M.E. Vazquez-Memije<sup>2</sup>, G. Chichierchia<sup>1</sup>, F.M. Santorelli<sup>1</sup>.* 1) Molecular Medicine Unit, Bambino Gesù Hosp, Rome, Italy; 2) Unidad de Investigación Genética Humana, Hospital de Pediatría, Centro Médico Nacional-IMSS, Mexico City, Mexico.

The F1F0 ATP Synthase is present in the bacterial plasma membrane, chloroplast thylakoid membrane and mitochondrial cristal membrane, this enzyme is responsible for most of the ATP cellular production. In mammals, two subunits of the F0 portion, A6 and A6L, are mtDNA encoded. Mutations in the ATPase 6 gene have been associated with MILS (maternally inherited Leigh syndrome) and with NARP (neuropathy, ataxia, retinitis pigmentosa). Evidence from family studies suggest that the T8993G (resulting in L156R) and T9176G (resulting in L217R) mutations result in more severe clinical phenotype than their allelic mutations, T8993C (L156P) and T9176C (L217P), respectively. To obtain clues as for the different pathogenic mechanisms leading to diverse clinical severity, we compared cellular ATP production, cell growth and entity of cellular response upon use of metabolic stressors (i.e., galactose, with or without oligomycin) in primary cell cultures obtained from patients harboring either the L156R, or L156P, or L217R, or L217P. We also analyzed clonal cells obtained after fusion of the abovementioned primary cultured cells with an osteosarcoma cell line, experimentally devoided of mtDNA. Clones harboring different percentage of mutated mtDNA molecules were selected for our studies. Our preliminary data suggest various levels of ATP production in the four cell lines, in agreement with the different clinical phenotype. Cell growth data showed a more severe effect in the mutations in which arginine substituted for leucine. The L156P mutation did not seem to be significantly affected after 5 days of treatment when galactose (+/- oligomycin) was substituted for glucose. This was not the case of L156R mutation.

**A mouse model of mitochondrial encephalopathy.** *W.J. Craigen, E. Weeber, M.J. Sampson, K. Anflous, M. Levy, D. Armstrong, J.D. Sweatt.* Dept Molec/Human Gen, Dept Path, and Div Neuroscience Baylor Col Medicine, Houston, TX.

The molecular basis for heritable mental retardation is poorly understood and undoubtedly multifaceted. In the case of mitochondrial disorders, mental retardation is common and not amenable to therapy. Possible mechanisms include reduced ATP production, alterations in calcium homeostasis, increased reactive oxygen species, or abnormal nitric oxide formation, amongst others. There is a paucity of animal models of mental retardation, regardless of the cause, yet animal models are needed to investigate and validate novel therapies for such complex phenotypes. We have generated mice lacking the major pathway across the mitochondrial outer membrane, the porin proteins VDAC1 and VDAC3, either as single locus or double locus mutants, and characterized the behavioral phenotypes of these mice. Two forms of learning were investigated using a standardized battery of tests; associative and spatial learning. All sensory parameters were normal. Each mouse strain exhibited distinct abnormalities in fear conditioning, a measure of associative learning, while all mutant mice failed to navigate the Morris water maze, reflecting defects in higher order learning and memory. To search for molecular correlates of these deficits, aspects of synaptic plasticity were measured in hippocampal slices using standard electrophysiologic protocols. Again, distinct abnormalities were found. By measuring paired pulse facilitation (PPF), VDAC3 deficient mice were demonstrated to have a pre-synaptic defect in neuronal plasticity. In contrast, VDAC1 deficient mice exhibited normal pre-synaptic function but abnormal post-synaptic function, as reflected in a reduction in the ability to generate long term potentiation (LTP). Double mutant slices demonstrated more pronounced defects in both PPF and LTP. These results demonstrate that there are distinguishable neuronal functions associated with each VDAC isoform, and that VDACS play an important role in learning, memory, and synaptic plasticity. These mice may serve as a model for mental retardation associated with mitochondrial dysfunction.

**Feasibility of using paraffin blocks for mitochondrial DNA mutation analysis.** *S.H. Kim<sup>1</sup>, S.H. Yoo<sup>1</sup>, J.G. Chi<sup>2</sup>*. 1) Dept.of Biology, College of Natural Sciences, Taegu Univ, Kyungsan 712-714, South Korea; 2) Dept.of Pathology, Seoul National University College of Medicine, Seoul 110-799, South Korea.

We investigated feasibility of using the formalin-fixed and paraffin-embedded tissue to study mitochondrial DNA mutations in the case that the fresh or frozen tissue, or blood samples are not available. 10 paraffin blocks of muscle biopsies in patients who had previously shown to have symptoms of mitochondrial disorder were chosen at random. These blocks had been processed between 1988-1996. Total DNA was extracted from these blocks and PCR amplification was carried out using the 24 standard DNA primer pairs for whole mitochondrial genome amplification used by other investigators and another primer pairs made by us. The size of the PCR product was between 800-1,600 bp. We could detect PCR products more than 800bp in length in many instances, although some investigators have reported the existence of limits to the size of fragments that can be amplified from such materials. The mitochondrial genome of our paraffin blocks is presumably in good condition (i.e. not much degraded than expected), since we could amplify up to 80% of whole mitochondrial genome in many cases. We could not confirm the presence of uncharacterized inhibitors of PCR reported by other investigators. The 3243 A to G MELAS mutation was confirmed in two PCR-amplified fragments by RFLP and sequencing. Our results are in accordance with the previous findings by other investigators that PCR allows molecular genetic analysis of paraffin-embedded tissues stored in most histopathology laboratories.

**Mitochondrial Myopathies: a South African perspective.** *A. Olckers<sup>1</sup>, D. Prosser<sup>1</sup>, D.C. Wallace<sup>2</sup>, M.D. Brown<sup>2</sup>, I. Smuts<sup>1</sup>.* 1) University of Pretoria, Pretoria, South Africa; 2) Emory University, Atlanta, USA.

Mitochondrial myopathies are the most frequent cause of metabolic abnormalities in the paediatric neurology population (Zeviani et al., 1996). Two recent studies estimate the prevalence of mitochondrial myopathies to be 1:15 000 in the adult English (Chinnery et al., 2000) and 1:11 000 in the paediatric Swedish (Darin et al., 2001) populations respectively. In this study a group of 42 paediatric patients who presented with phenotypes suspected to be due to a mitochondrial aetiology, were included. Patients were screened for ten of the most frequent mtDNA mutations that cause MELAS, MERRF or Leigh Syndrome. RFLP or Single Stranded Sequence analyses were utilised to detect the presence of the respective mutations. In 21 patients all twenty-two mitochondrial tRNA encoding genes were sequenced. Full-length mtDNA sequences were generated for six patients. The presence of one reported mutation (A3243G) has been verified in a single proband. Upon investigation this mutation was also detected in her mother and sister, but was found to be absent in her other maternal relatives. In the three individuals harbouring this mutation different levels of heteroplasmy was observed. To date, none of the other reported mtDNA mutations have been observed in this group of South African paediatric patients who have been well characterised on the clinical level.

**Antenatal manifestations of mitochondrial respiratory chain deficiency.** *J.C. Von Kleist-Retzow<sup>1,2</sup>, P. de Lonlay-Debeney<sup>1</sup>, V. Cormier-Daire<sup>1</sup>, G. Viot<sup>1</sup>, J. Amiel<sup>1</sup>, P. Rustin<sup>1</sup>, A. Rötig<sup>1</sup>, A. Munnich<sup>1</sup>.* 1) INSERM U393, Hosp Necker, Paris, France; 2) Pediatric Clinic, University Cologne, Germany.

Antenatal manifestations of respiratory chain deficiency have not been hitherto described. Reviewing the clinical features of 300 patients with proven respiratory chain deficiency, we found that 20% had intrauterine growth retardation (IUGR). Most children were born at (or close to) term and macrosomia, hydramnios or oligoamnios with fetal immobility were rarely noted. Hydrops fetalis was frequently observed during pregnancy of children who further developed Pearson syndrome. Interestingly, a number of malformations of various types were noted namely cerebral malformations (agenesis or hypoplasia of the corpus callosum, cerebellar hypoplasia), gastrointestinal malformations (duodenal atresia, duplication of the choledochus, agenesis of the gallbladder), renal malformations (dilatation of pyelocalicial cavities and the bladder) and craniofacial dysmorphism. Round face, high forehead, flat philtrum, low set ears, short neck, ear dysplasia characteristic for CHARGE association and an incomplete VATER association were occasionally observed in our series. Other malformations included cardiomegaly and supraventricular tachycardia in the third trimester. Similarly, echo doppler may occasionally provide important information. Indeed, a boy with complex I deficiency had IUGR, hypertrophic cardiomyopathy and Leigh syndrome in his first year of life. Retrospective analyses revealed the presence of enlarged ventricles with bilateral porencephalic cysts in the germinal matrix and reduced cerebral blood flow in the brainstem consistent with vascular injury at 22 weeks of gestation. This study suggests that the antenatal manifestations of respiratory chain deficiencies have been largely overlooked and give strong support to the early intrauterine expression of the disease-causing genes in mitochondrial diseases.

Program Nr: 1809

**Molecular analysis of the SURF-1 gene associated with Leigh syndrome within the South African paediatric population: a pilot study.** *D. Prosser<sup>1</sup>, I. Smuts<sup>1</sup>, M.D. Brown<sup>2</sup>, D.C. Wallace<sup>2</sup>, A. Olckers<sup>1</sup>.* 1) University of Pretoria, South Africa; 2) Emory University, Atlanta, USA.

Leigh syndrome (LS) is a devastating neurological disorder which has onset during the first two years of life. The clinical manifestations of LS include psychomotor regression, brainstem abnormalities and lactic acidosis. To date three modes of inheritance have been described to be associated with LS. Maternal inheritance with mutations in the ATP6 gene of the mitochondrial genome, X-linked inheritance associated with mutations in the PDHA1 gene encoding the E1a subunit of the pyruvate dehydrogenase complex and autosomal inheritance. The latter is associated with mutations in the SURF-1 gene, localised to chromosome 9q34. The SURF-1 protein is responsible for maintenance of cytochrome c oxidase (Tiranti et al., 1998, and Zhu et al., 1998) Twelve patients diagnosed on both a clinical and biochemical level were included in this study. The mtDNA mutations T8993G/C and T9176C have been excluded as the cause of the phenotype observed in these patients. The SURF-1 analysis was performed via PCR of the nine exons, followed by SSCP and Heteroduplex (HEX) analyses. Aberrant fragments observed via SSCP-HEX were investigated via single stranded sequence analyses.

**Studies of pathogenesis in Farber disease.** *T. Muramatsu*<sup>1</sup>, *Y. Yamada*<sup>1</sup>, *N. Sakai*<sup>1</sup>, *H. Tsukamoto*<sup>1</sup>, *K. Isahara*<sup>2</sup>, *Y. Uchiyama*<sup>2</sup>, *K. Inui*<sup>1</sup>. 1) Dept of Pediatrics, Osaka Univ, Suita, Osaka, Japan; 2) Dept of Anatomy, Osaka Univ, Suita, Osaka, Japan.

Farber disease is a rare autosomal recessive sphingolipidosis caused by a deficiency of acid ceramidase, a lysosomal enzyme that normally catalyzes the hydrolysis of ceramide to sphingosine and free fatty acids. The disease is clinically characterized by swollen joints with limitation of movements, disseminated subcutaneous nodules and progressive cachexia. The storage material, ceramide, has attracted a great attention as an intracellular effector molecule in apoptosis. In this study we measured inflammatory cytokines in patients plasma and histologically examined the nodules. We found the slight increase of IL-6 but normal level of TNF-, and TUNEL positive staining cells in the nodules. The cells were co-stained with activated caspase-3 and CD68, and were identified as macrophages. The apoptotic changes were due to the activation of caspase 3 systems. We also screened gene expression in subcutaneous nodules and found expression several genes including caspase3, TGF-1, THF-, and MCP-1. Using cell lines derived from macrophage, mRNA expressions of NF-kB, TNF- and MCP-1 were increased with dose dependently by C2-ceramide addition into the culture medium. In Farber disease ceramide accumulation occur in lysosome and some ceramide escape to cell membrane, which induces production of some cytokines and apoptotic changes in macrophages. These events may partly explain the pathogenesis of Farber disease by the ceramide function as a second messenger.



**Expression of Arginase Isozymes in Mouse Brain.** *H. Yu*<sup>1,2</sup>, *R.K. Iyer*<sup>1,3</sup>, *R. Kern*<sup>2</sup>, *W. Rodriguez*<sup>1,2</sup>, *W.W. Grody*<sup>1,3,4</sup>, *S.D Cederbaum*<sup>1,2,4</sup>. 1) Mental Retardation Research Center; 2) Dept of Psychiatry; 3) Dept of Pathology and Laboratory Medicine; 4) Dept of Pediatrics; UCLA School of Medicine, Los Angeles, CA.

The two forms of arginase in man, identical in enzymatic function, are encoded in separate genes and are expressed differentially in various tissues. AI is expressed predominantly in the liver cytosol and is thought to function primarily to detoxify ammonia as part of the urea cycle. AII, in contrast, is predominantly mitochondrial and is more widely expressed, and is thought to function primarily, to produce ornithine. Ornithine is a precursor in the synthesis proline, glutamate and polyamines. Both forms of arginase are expressed at similar levels in both human and mouse brain. This study was undertaken to explore the cellular and regional distribution of AI and AII expression in brain using immunohistochemistry and in situ hybridization. It is the first step in a longer-term project to explore the perturbations of AII in the AI knockout mouse and those of AI in the AII knockout animal, and the biochemical implications of hyperargininemia on neural cell injury and apoptosis, nitric oxide synthesis and excitotoxic injury. AI and AII are detected only in neurons and are generally co-expressed in most cells studied. Expression is particularly high in cerebellar, brainstem and spinal cord neurons. Minor regional differences in AI and AII distribution may exist but are not dramatic. GAD (glutamic acid decarboxylase) 65 and GAD 67, postulated to be related to the risk of glutamate excitotoxic injury are similarly ubiquitous in their expression and generally parallel arginase expression patterns. Neither AI nor AII was seen in astrocytes, oligodendrocytes or white matter. Understanding differential arginase expression in brain is critical to understanding the function of the two isozymes, the pathogenesis of human AI deficiency and hence its treatment. The co-expression of the two isozymes, if present in different cellular compartments, may not necessarily represent overlapping and hence redundant function. The AI knockout mouse should provide an important additional tool in our quest and allow a more thorough dissection of the function of each isozyme.

***SUTAL* gene mutations causing Hermansky-Pudlak Syndrome Type-3 in a non-Puerto Rican family.** P. Anderson, D.L. Fitzpatrick, M. Huizing, Y. Anikster, W.A. Gahl. Section on Human Biochemical Genetics, HDB, NICHD, NIH, Bethesda, MD.

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder characterized by oculocutaneous albinism and a storage pool deficiency due to absence of platelet dense bodies. Lysosomal ceroid lipofuscinosis, pulmonary fibrosis, and granulomatous colitis are occasional manifestations of the disease. Patients can have HPS due to mutations in one of several different genes. *HPS1* mutations cause HPS-1 disease, with a relatively severe phenotype and a risk of pulmonary fibrosis. *ADTB3A* mutations cause HPS-2 disease, with relatively mild disease plus neutropenia. HPS-3 disease is caused by mutations in *SUTAL*, isolated after linkage mapping in a genetic isolate of mildly affected HPS patients from central Puerto Rico. *SUTAL* is a 3015-bp gene coding for a 113.7 kD protein of unknown function. Central Puerto Rican HPS-3 patients are homozygous for a 3094-bp deletion that involves exon 1 and part of intron 1 of *SUTAL*, plus 2874-bp of upstream sequence. We screened non-Puerto Rican HPS patients for *SUTAL* mutations by northern blot analysis and cDNA sequencing. In one 5-year old boy with mild oculocutaneous albinism and a minimal bleeding diathesis, we found a heterozygous C1329T (Arg/Trp) mutation in exon 6, in addition to a splice site mutation (G2729+1C) causing deletion of exon 14. Screening of family members of the HPS proband revealed that the proband's affected sister also possessed the C1329T mutation, as did his unaffected father. The proband's unaffected sister, half-sister and mother did not possess the C1329T mutation. The splice site mutation was found in the mother, both sisters and the half sister, but not the father. This case illustrates the occurrence of HPS-3 in a non-Puerto Rican family presenting with mild disease, and segregation of *SUTAL* mutations with the disease phenotype. It emphasizes the importance of screening non-Puerto Rican patients with Hermansky-Pudlak syndrome for *SUTAL* mutations other than the founder mutation present in central Puerto Ricans.

***PEX6* Mutation Identification in 20 Patients with Peroxisome Biogenesis Disorders.** *N.E. Braverman<sup>1</sup>, C. Obie<sup>1</sup>, P. Lin<sup>1</sup>, D. Valle<sup>1</sup>, A.B. Moser<sup>2</sup>, H.W. Moser<sup>2</sup>, S.J. Steinberg<sup>2</sup>.* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Univ, Baltimore, MD; 2) Dept of Neurogenetics, Kennedy Krieger Institute, Baltimore, MD.

Peroxisome biogenesis disorders (PBD) are a heterogeneous group of autosomal recessive diseases associated with failure to assemble mature peroxisomes. The patients are divided into 12 complementation groups (CGs) based on recovery of peroxisomal functions after cell fusion. Each CG reflects a defect in a different *PEX* gene. 80% of PBD patients belong to CGs affecting *PEX1* (CG1) and *PEX6* (CG4). *PEX1* and *PEX6* proteins are interactive, partially redundant AAA ATPases whose role in peroxisomal matrix protein import involves recycling of the receptors. To further characterize these proteins and to determine phenotype-genotype relationships, we performed a comprehensive mutation analysis in CG4 probands.

*PEX6* has a 2.9 kb ORF encoded by 17 exons that span 16kb of genomic DNA on 6p21.1. We amplified genomic DNA by multiplex PCR and directly sequenced the amplicons. This process identified mutations in 31/40 alleles (77.5%). We found 16 missense mutations, 2 small insertions, 3 single base pair deletions and 10 large intragenic deletions that involve direct repeat sequences. The majority of the mutations were unique. We did ASO analysis to determine the frequency of the missense alleles in the general population. None were present except one, P285A, which was found once in 150 control genes. In addition, we identified six polymorphic changes, the most common were V133V (19 alleles) and P939Q (14 alleles). Consistent with results from other PBD CGs, alleles predicted to have the most severe effect on protein function were associated with Zellweger syndrome, the most severe phenotype.

The high frequency of CG1 is due partly to the presence of 2 *PEX1* founder alleles. However, the high frequency of CG4 cannot be explained this way. Interestingly, one of our probands is cousin to a patient in CG1. We propose that some CG4 patients might be compound heterozygotes for defects in both *PEX6* and *PEX1*.

**A common mutation and a novel mutation in the *ORNT1* gene of Japanese patients with mitochondrial ornithine transporter deficiency (HHH syndrome).** *T. Miyamoto*<sup>1</sup>, *N. Kanazawa*<sup>1</sup>, *S. Kato*<sup>2</sup>, *M. Kawakami*<sup>3</sup>, *T. Inoue*<sup>4</sup>, *K.*

*Takeshita*<sup>5</sup>, *I. Yoshida*<sup>6</sup>, *C. Hayakawa*<sup>7</sup>, *S. Tsujino*<sup>1</sup>. 1) Inherited Metabolic Diseases, National Inst Neuroscience, Tokyo, Japan; 2) Department of Internal Medicine, Shinfuji hospital, Shizuoka, Japan; 3) Department of Neurology, St. Marianna University School of Medicine, Kanagawa, Japan; 4) The Department of Pediatrics; Western Shimane Medical and Welfare Center for the Handicapped; Gohtsu, Japan; 5) Division of Child Neurology; Institute of Neurological Sciences; Faculty of Medicine; Tottori University; Yonago, Japan; 6) Department of Medical Education and Pediatrics, Kurume University School of Medicine, Fukuoka, Japan; 7) Department of Pediatrics, Central Hospital, Aichi Welfare Center for Persons with Developmental Disabilities, Aichi, Japan.

Mitochondrial ornithine transporter Deficiency (HHH syndrome) presents with various neurological symptoms, including mental retardation, spastic paraparesis with pyramidal signs, cerebellar ataxia. We previously described three mutations (R179X, G27E, and 228/229 insAAC) in the *ORNT1* gene in three Japanese patients with HHH syndrome. In this report, four additional Japanese patients (52-year-old woman, 10-year-old man, 15-year-old man, and 46-year-old woman; ages are at diagnosis) were tested. At first, we screened these patients for the three mutations with the PCR-RFLP or ARMS method as described. As a result, 3 of the 4 patients were homozygous for R179X. Together with our previous results, 4 of 7 Japanese patients (8 of 14 alleles) had R179X, suggesting that this is a common mutation in Japanese patients with HHH syndrome. Therefore, detecting this mutation may be feasible for diagnosis in Japanese patients. As one patient had none of the three mutations, we amplified exonic fragments containing the entire coding region of the *ORNT1* gene by PCR with intronic primers, and revealed a novel mutation, C-to-G transition resulting in a change of CCC to CGC at codon 126 (P126R) in exon 3. The patient was homozygous for P126R. Mutations so far reported in the *ORNT1* gene are distributed throughout most exons, and there appears to be no "hot spot" for mutations.

**Identification of novel mutations and three most common mutations in the human ATP7B gene of Korean patients with Wilson disease.** *G.H. Kim, E.J. Seo, H.W. Yoo.* Medical Genetics Clinic & Laboratory, Asan Medical center, Ulsan University College of Medicine, Seoul, Korea.

Wilson disease is an autosomal recessive disorder of copper transport, which is probably the most common inherited metabolic disorder in Korea. In Wilson disease, synthesis of a defective copper transporting enzyme leads to the accumulation of copper in the liver, brain and kidney. The product of the Wilson disease gene is a copper transporting P-type ATPase(ATP7B). In this study, efforts have been made to identify novel mutations and investigate a frequency of the common mutations in Korean patients with Wilson disease. This study includes 37 patients from 33 unrelated Korean families with Wilson disease. Genomic DNA from peripheral leukocytes or skin fibroblasts and cDNA from liver tissue were PCR amplified exon by exon, subsequently analyzed using heteroduplex or SSCP analysis. Specimen showing mobility shift on those studies were directly sequenced. We identified 12 different mutations in 33 Korean families with Wilson disease; Arg778Leu(R778L), Asn1270Ser(N1270S), Ala874Val(A874V), 2304 del C, 27bp deletion in exon 11, 2461 ins C, Cys656Stop(C656X), Pro768His(P768H), Leu1083Phe(L1083F), Ala1168Ser(A1168S), Leu1255Ile(L1255I), and Asp1267Ala(D1267A). Among these, 6 mutations(27 bp deletion in exon 11, 2461 ins C, C656X, P768H, A1168S, and L1255I) are novel, the R778L mutation has been known to be highly prevalent in Asian patients. The allele frequency of the R778L in Korean patients with Wilson disease was 37.9%, which was slightly higher than those of Japanese and Taiwanese. Interestingly, the N1270S, originally described in an Italian patient, was the next common mutation in Korean patients with Wilson disease with the allele frequency of 12.1%, which was presumed to disrupt ATP hinge domain of the ATP7B protein. The A874V mutation was the thirdly common mutation with the allele frequency of 9.4%, which was presumed to disrupt Td domain of the ATP7B protein. R778L, N1270S, and A874V mutation are three major mutations covering upto nearly 60% of mutated alleles, though Korean patients with Wilson disease are genetically heterogeneous.

**The *CTNS* promoter and its mutations causing cystinosis.** C. Phornphutkul, Y. Anikster, M. Huizing, J. Chou, W. Gahl. HDB, NICHD, NIH, Bethesda, MD.

Cystinosis is a rare autosomal recessive storage disease resulting from impaired transport of the disulfide amino acid cystine out of cellular lysosomes. The cystinosis gene, *CTNS*, contains 12 exons and codes for a 367-amino acid peptide that appears to be an integral membrane protein functioning as the lysosomal cystine transporter. Although more than 55 mutations in the *CTNS* coding sequence have been reported, no regulatory mutations have been found because the promoter region has not been identified. By using CAT (chloramphenicol acetyltransferase) reporter constructs of sequences 5' to the *CTNS* coding sequence, we identified the *CTNS* promoter as the region from nucleotides -316 to +1 with respect to the transcription start site. Several predicted transcription factors exist in this region, including an Sp-1 motif at nucleotide -299 to -293, verified by gelshift assays. In studying patients who were not homozygous for the common 57-kb deletion in *CTNS*, we identified three patients who exhibited mutations in the *CTNS* promoter region. All three had only one mutation in the *CTNS* coding sequence. One nephropathic cystinosis patient carried a -295 G->C substitution that disrupted the Sp-1 motif, while two patients with ocular cystinosis displayed a -303 G->T substitution and a -303 T insertion, respectively. Each mutation drastically reduced CAT activity when inserted into a reporter construct. Moreover, each failed to cause a mobility shift when exposed to nuclear extract or to compete with the normal oligonucleotide's mobility shift. On both clinical and functional grounds, all three mutations are considered severe, explaining the patients' clinical manifestations. The *CTNS* promoter also shared 41 nucleotides with the promoter of an adjacent gene of unknown function, *CARKL*, whose start site is 501 base pairs from the *CTNS* start site. However, the patients' *CTNS* promoter mutations had no effect on *CARKL* promoter activity. These findings suggested that the *CTNS* promoter region should be examined in cystinosis patient having fewer than two coding sequence mutations.

**Congenital erythropoietic porphyria: Identification, characterization and genotype/phenotype correlations of novel gene mutations.** A.A. Shady, B. Colby, K.H. Astrin, R.J. Desnick. Human Genetics, Mount Sinai School of Medicine, New York, NY.

Congenital erythropoietic porphyria (CEP) is an autosomal recessive disorder which results from the markedly deficient activity of uroporphyrinogen III synthase (UROS), the fourth enzyme in the heme synthetic pathway. Clinical manifestations range from hydrops fetalis to lifelong transfusion dependency to mild cutaneous photosensitivity. To investigate the genetic heterogeneity underlying CEP, we determined the UROS mutations in seven unrelated patients whose clinical manifestations varied from moderate to severe. All 10 exons, the adjacent intron/exon boundaries, and the housekeeping and erythroid promoters of the UROS gene were PCR-amplified and sequenced on an ABI Prism 377 sequencer. Three previously documented mutations (C73R, T228M and -86C→A) and seven novel mutations were detected: four missense (A69T, E81D, G188W and I219S), one single-base deletion (21G), a large 28-base insertion (672ins28), and one complex mutation (6276ins39). While both E81D and G188W are adjacent to the exon/intron boundary, RT-PCR analysis showed that only E81D altered splicing by deleting exon 4. Each novel missense mutation was expressed in *E. Coli*, and its residual enzymatic activity was determined. With the exception of E81D, residual enzymatic activity was markedly reduced for the A69T, G188W and I219S missense mutations. Expression of the E81D allele showed relatively high residual activity; however, it was less heat stable than the wild type ( $T_{1/2}$  at 37°C was 40 min vs. 90 min, respectively.) The total enzyme activity from both alleles in each patient correlated with disease severity. These findings document the molecular heterogeneity and provide genotype-phenotype correlations in CEP.

**Hypoglycemia and dicarboxylic aciduria as the presenting biochemical findings in a patient with Barth syndrome.** *D.J. Waggoner, R.L. Brown, J. Hedrick.* University of Chicago, Department of Human Genetics, Chicago, USA.

Barth syndrome is a rare but well described X-linked metabolic disorder characterized by cardiomyopathy, neutropenia, growth retardation, skeletal myopathy, and 3-methylglutaconic aciduria. Affected males usually die due to complications of cardiomyopathy in the first year of life; although phenotypic variability exists and there are reports of males who have survived into adulthood. The gene responsible for Barth syndrome, G4.5, was reported in 1996 and encodes a novel protein whose function remains unknown. Studies have been unable to show a genotype/phenotype correlation with the various mutations reported. Barth syndrome is suspected in patients with 3-methylglutaconic aciduria, cardiomyopathy, and neutropenia but the differential diagnosis includes defects of the mitochondrial respiratory chain. Patients with mitochondrial dysfunction typically have additional metabolites, which serve to differentiate them. We report an 11-month-old boy with a history of hypotonia who presented in heart failure secondary to cardiomyopathy and was found to have significant hypoglycemia and acidosis. There was no history of increased infections or neutropenia. Initial results of urine organic acid studies showed 3-methylglutaconic acid, large amounts of C6-C10 saturated and unsaturated dicarboxylic acids and 3-hydroxycarboxylic acids. The patient was eventually found to have the deleterious mutation Arg93Gly in the G4.5 gene confirming the diagnosis of Barth syndrome. These findings broaden the biochemical presentation of patients with Barth syndrome to include hypoglycemia and dicarboxylic aciduria often seen as the presenting biochemical findings in fatty acid oxidation disorders or mitochondrial defects. Molecular testing for mutations in G4.5 is a valuable component of the evaluation of these complicated patients with multiple diagnostic possibilities.



**Hyperoxaluria type 1: a novel 2 bp deletion in AGXT resulting in loss of the peroxisomal targeting signal.** *M.B. Coulter-Mackie<sup>1,2</sup>, J.R. Toone<sup>1,2</sup>, D.A. Applegarth<sup>1,2,3</sup>*. 1) Department of Pediatrics, University of British Columbia, Vancouver, BC; 2) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC; 3) Biochemical Diseases Lab, B.C. Children's Hospital, Vancouver, BC.

Primary hyperoxaluria type I, PH1, results from a deficiency of alanine glyoxylate aminotransferase (AGT) activity. AGT is a peroxisomal enzyme in humans. A common polymorphism, P11L, provides an N-terminal mitochondrial targeting signal in the so-called minor allele. The most common PH1 mutation, G170R, when in *cis* with P11L, results in mis-targeting of AGT to mitochondria. A patient with pediatric onset PH1 was assessed biochemically and at the level of her DNA. Her liver AGT enzymic activity was 4.7% of normal controls but she had an almost normal level of AGT-specific CRM at 80% compared to normal liver. DNA analysis demonstrated that she was homozygous for the major allele indicating she was not a candidate for mis-targeting. She was found to carry one copy of the mutation, G82E, on her maternal allele. This is a previously documented recurrent mutation reported to result in a normally-targeted inactive protein. On the paternal allele she carried a 2 bp deletion in the final exon of the AGT gene. Because the resultant frameshift occurs in the last exon, it is expected that the allele would be translated. Such an allele, along with the G82E allele, would account for the high level of immunoreactive material. However, the C-terminal peroxisomal targeting signal would be lost from the protein product of the deletion allele. The AGT produced from this allele would un-targeted rather than mis-targeted and would be expected to remain in the cytoplasm.

This work was supported in part by a grant from the Garrod Association of Canada.

**Intermediate phenotype of rhizomelic chondrodysplasia punctata.** *P.K. Chakraborty<sup>1</sup>, N. Braverman<sup>2</sup>, J.T.R.C. Clarke<sup>1</sup>.* 1) Department of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada; 2) McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins Medical Center, Baltimore, MD.

Rhizomelic chondrodysplasia punctata (RCDP) is a genetically heterogeneous disorder that classically presents with congenital cataracts, rhizomelia, epiphyseal calcifications, contractures, dysmorphic facial features, and severe psychomotor and growth retardation with early demise. We present the case of a 3-year-old boy with an intermediate clinical and biochemical phenotype of RCDP. He had congenital cataracts surgically removed at 1 month of age. At 20 months of age, multiple joint contractures and epiphyseal stippling were noted. He is not rhizomelic, but has mild facial dysmorphisms seen in patients with RCDP including a Binder's nose and prominent forehead. He was slightly delayed in reaching early milestones, and now has mild global delay with speech being most affected. Investigations of peroxisomal function demonstrate defects of phytanic acid catabolism and plasmalogen synthesis, with normal very long chain fatty acid and cholesterol synthesis. Plasma phytanic acid is markedly elevated at 365 mM, while RBC plasmalogens are moderately decreased (C16:0 DMA / C16:00 fatty acid = 0.031 [normal control: 0.051-0.09, RCDP control: 0.001-0.05], C18:0 DMA / C18:00 fatty acid = 0.109 [normal control: 0.137-0.255, RCDP control: 0.001-0.05]). Skin fibroblast peroxisomal plasmalogen synthesis (3H/14C incorporation = 3.037 [mean normal control: 0.67, mean RCDP control: 33.67]) is intermediately impaired. Fibroblast phytanic acid oxidation (18% control) is in the range of classical RCDP. The biochemical profile in this patient is characteristic for defects in the peroxisome receptor, Pex7. Pex7 targets a subclass of matrix enzymes to the peroxisome, including phytanyl CoA hydroxylase (PAHX) and alkyl-dihydroxyacetone phosphate synthase. Only a small number of mild RCDP variants are reported and the clinical spectrum is unknown. In all of these, plasmalogen biosynthesis is moderately affected, but PAHX activity remains severely defective. These patients support a major role for plasmalogens in determining the RCDP phenotype. PEX7 mutation analysis for this patient is in progress.

**Peroxisomal biogenesis: Study of protein-protein-interactions of early peroxins by fluorescence resonance energy transfer (FRET) analysis.** *A.C. Muntau<sup>1</sup>, A.A. Roscher<sup>1</sup>, W.-H. Kunau<sup>2</sup>, G. Dodt<sup>2</sup>.* 1) Dr. von Hauner Children's Hospital, Clinical Chemistry and Biochemical Genetics, Ludwig-Maximilians-University, Munich, Germany; 2) Physiological Chemistry, Ruhr-University, Bochum, Germany.

Peroxisomal biogenesis disorders (PBD) are characterized by an impairment of multiple peroxisomal functions phenotypically presenting as Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum disease or rhizomelic chondrodysplasia punctata. Proteins involved in peroxisomal assembly are termed peroxins and are encoded by *PEX* genes. Organelle biogenesis requires the formation of a lipid bilayer, the insertion of membrane proteins, and the import of matrix proteins. Defects in most *PEX* genes result in impaired peroxisomal matrix protein import, whereas the ability to import membrane proteins is often retained. Fibroblasts from patients with defects in *PEX3*, *PEX16* or *PEX19*, however, display a cellular phenotype characterized by a lack of even residual peroxisomal membrane structures. A protein-interaction between *PEX3* and *PEX19* has been shown to be a crucial functional component of early peroxisomal assembly. However, the intracellular site of this interaction is still unclear.

To address this issue by fluorescence resonance energy transfer (FRET) analysis, we tagged *PEX3* and *PEX19* to yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP), respectively. Coexpression of fully functional *PEX3*-YFP and *PEX19*-CFP fusion proteins in *PEX3*-deficient fibroblasts revealed a FRET signal not only at the peroxisomal membrane, but also in the cytosol. Control coexpression of *PEX3*-YFP and CFP-N1 yielded only a weak background signal. Our data show that FRET analysis is able to reveal quantitative spatio-temporal information about the physical interaction of peroxins during the process of peroxisomal assembly in single living cells. This may contribute to unravel some basic aspects of peroxisome biogenesis.

**Molecular diagnosis of Refsum Disease, a disorder of the peroxisomal phytanic acid  $\alpha$ -oxidation.** *G.A. Jansen<sup>1</sup>, H.R. Waterham<sup>1</sup>, D.M. van den Brink<sup>1</sup>, S. Ferdinandusse<sup>1</sup>, E.M. Hogenhout<sup>1</sup>, O.H. Skjeldal<sup>2</sup>, O. Stokke<sup>3</sup>, R.J.A. Wanders<sup>1</sup>.* 1) Dept. Clinical Chemistry, Laboratory for Genetic Metabolic Diseases, Academic Medical Centre, University of Amsterdam, The Netherlands; 2) Dept. Pediatrics, Neurological section, Rikshospitalet, Oslo, Norway; 3) Institute of Clinical Chemistry, Rikshospitalet, Oslo, Norway.

Refsum disease is a rare inherited disorder of lipid metabolism with retinitis pigmentosa, peripheral neuropathy, cerebellar ataxia and elevated protein levels in the cerebrospinal fluid as main manifestations. Patients with Refsum disease accumulate phytanic acid, an unusual branched chain fatty acid (3,7,11,15-tetramethylhexadecanoic acid) in tissues and body fluids, which is the biochemical hallmark of this disorder used to confirm the clinical diagnosis. The accumulation of phytanic acid is the result of a deficient activity of phytanoyl-CoA hydroxylase (PhyH), a peroxisomal enzyme which catalyses the conversion of phytanoyl-CoA to 2-hydroxyphytanoyl-CoA, one of the four steps of the phytanic acid  $\alpha$ -oxidation degradation pathway. PhyH deficiency is caused by mutations in *PHYH*, the gene encoding this enzyme. Until recently, only cDNA mutation analysis was available as a tool for diagnosing the genetic defect in Refsum disease. After resolving the structure of the *PHYH* gene, we now have developed a method for rapid mutation analysis at the genome level, using the PCR technique to amplify the nine exons of the 21 kb *PHYH* gene in eight fragments, followed by sequence analysis. Using this method, we have analysed genomic DNA from a large number of Refsum patients, and have found more than 20 different mutations, including missense mutations, insertions, deletions, some of which lead to missplicing events. Most mutations were located in exons six and seven of the *PHYH*-gene, but the most frequent mutation with a allele frequency of about 20% was present in the splice acceptor site of IVS2, resulting in skipping of exon 3.

**Large 5' deletions in the X-linked adrenoleukodystrophy gene, *ABCD1*, in two patients with a novel neonatal phenotype.** *S.J. Steinberg*<sup>1</sup>, *D. Corzo*<sup>2</sup>, *W.T. Gibson*<sup>3</sup>, *G.A. Mitchell*<sup>3</sup>, *G. Cox*<sup>2</sup>, *G. Cutting*<sup>4</sup>, *C. Boehm*<sup>4</sup>, *H. Tyson*<sup>4</sup>, *P.A. Watkins*<sup>1</sup>, *G.V. Raymond*<sup>1</sup>, *A.B. Moser*<sup>1</sup>, *H.W. Moser*<sup>1</sup>. 1) Kennedy Krieger Inst, Baltimore, MD; 2) Children's Hosp, Boston, MA; 3) Medical Genetics Service, Ste-Justine Hosp, Montreal, Canada; 4) Johns Hopkins Univ, Baltimore, MD.

Adrenoleukodystrophy (X-ALD) is caused by mutations in the ALD gene, *ABCD1*, which encodes a peroxisomal integral membrane protein (ALDP). All previously described X-ALD patients have been asymptomatic at birth, the earliest onset of neurologic symptoms being 2.75 years. We now report the molecular genetic basis of X-ALD in two patients with a presentation more typical of a peroxisomal biogenesis disorder. Both were hypotonic and had cholestatic liver disease. In addition, Patient (Pt) 1 (Corzo, D et al., *Am J Hum Genet* 65, 4 (suppl):A1295) had sensorineural hearing loss, seizures and died at 4 months. Pt 2 had bilateral polar cataracts, neurological deterioration and died of failure at 12 months. Plasma very long chain fatty acids were characteristic of a peroxisomal  $\beta$ -oxidation defect in both patients. Additional biochemical studies in blood and cultured cells did not reveal additional peroxisomal metabolic defects. Immunofluorescence microscopy of cultured fibroblasts from these patients showed that they had normal appearing peroxisomes that lacked ALDP. Based on these results, mutation analysis of all 10 *ABCD1* exons was initiated by PCR and direct sequencing of genomic DNA. Exon 1-5 failed to amplify in Pt 1 and exons 1-10 failed to amplify in Pt 2. Southern blot analysis confirmed the absence of these exons, but the precise breakpoints have not been defined. Of 230 non-recurrent *ABCD1* mutations 5% involve a deletion of at least 1 exon. Only one previously reported patient had a deletion involving exon 1, a 0.5kb deletion at the 3' end of the 1.286kb exon. Further studies to determine if the deletions in these boys extend beyond the 5' end of exon 1 are in progress. Once the deletion breakpoints are defined we can initiate investigations that would shed light on the pathogenetic mechanism of this new X-ALD phenotype and perhaps on the function of ALDP itself.

**Genetic Variation in the Human Organic Cation Transporter hOCT2.** *M.K. Leabman<sup>1</sup>, J. DeYoung<sup>2</sup>, C. Huang<sup>3</sup>, S.J. Johns<sup>3</sup>, K.M. Giacomini<sup>1</sup>*. 1) Department of Biopharmaceutical Sciences, School of Pharmacy, University of California, San Francisco, San Francisco, CA; 2) Neurogenetics Laboratory, Program in Human Genetics, University of California, San Francisco, San Francisco, CA; 3) Sequence Analysis and Consulting Service, Computer Graphics Laboratory, Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA.

Membrane transporters play a critical role in drug response, affecting the pharmacokinetics and/or pharmacodynamics of drugs. The organic cation transporter, hOCT2, is thought to play a major role in the renal elimination of a variety of structurally diverse basic drugs (e.g., cimetidine, metformin). Therefore, genetic variation in this transporter may contribute to variability in renal clearance. We recently conducted denaturing HPLC analysis followed by automated sequencing on an ethnically diverse, 250 sample collection of genomic DNA to identify variants of hOCT2. Twenty eight variants, 16 coding and 12 non-coding, were identified and their frequencies in each ethnic population were determined. Three common, non-synonymous coding region variants have been constructed and functional studies in *Xenopus laevis* oocytes have shown that they transport the prototypical organic cation MPP<sup>+</sup>. In RNA dose response studies, the uptake of a saturating concentration of MPP<sup>+</sup> in variant 1 and variant 3 expressing oocytes was lower than that of wild type at all doses of RNA ( $p < 0.05$ ), suggesting that the  $V_{max}$  of MPP<sup>+</sup> in the variant expressing oocytes differs from that in oocytes expressing the wild type transporter. Potency of inhibition of MPP<sup>+</sup> uptake of tetrabutylammonium (TBA) differed in oocytes expressing two of the variants. These data demonstrate that the three common polymorphisms of hOCT2 are functional, but differ from the wild type hOCT2 in terms of their kinetic characteristics and interactions with hydrophobic organic cations.

This work was supported by a grant from the National Institutes of Health (GM61390).

**Characterization of a carbohydrate response element (ChoRE) regulating the gene for human galactose-1-phosphate uridylyltransferase (hGALT).** *L.J. Elsas II, A. Webb, S.D. Langley.* Dept Pediatrics, Medical Gen, Emory Univ, Atlanta, GA.

We previously found that D-galactose stimulated hGALT in HepG2 and OVCAR-3 cells and that a four bp deletion (-119 to -116delGTCA) in the hGALT promoter region was linked to the Duarte mutation in galactosemia, disrupted the spatial relationship between two E-box motifs (CANNTG) and reduced a positive regulatory response (*Molec. Genet. Metab.* 27:297-305, 2001). In the wild type promoter there are three sequential GTCA repeats in this positive regulatory domain. Here we define the effects of altering the 22 bp spacing and number of GTCA repeats between the -145 to -140 CAGGTG and -117 to -112 CACGTG E-box motifs on *trans* nuclear protein binding and function. We compare electrophoretic mobility shift assays (EMSA) and dual luciferase reporter constructs transfected into HepG2 cells. Radiolabeled oligonucleotide probes representing wild type (WT)(38 bp), WT-GTCA (34 bp) and WT+GTCA (42 bp) encompassed both E-box motifs and specifically bound c-myc and at least one other nuclear protein. Unexpectedly, the 42 bp probe bound more *trans* factor ( $1010 \pm 50$ ) than the WT 38 bp probe ( $450 \pm 29$ ) and the WT-GTCA probe bound the least ( $360 \pm 32$  CPM) ( $p < 0.05$ ). These structural binding studies correlated with functional studies of hGALT promoter constructs containing the WT, WT-GTCA and WT+GTCA sequences where dual luciferase ratios were  $5.8 \pm 2$ ,  $3.6 \pm 1$  and  $12 \pm 2$ , respectively. When HepG2 cells were incubated in 5mM galactose before and during transfection, promoter activity increased ten-fold. We conclude that the hGALT promoter contains a ChoRE contained within two E-box motifs whose spatial relationship determines the degree of positive response to galactose stimulation by specific *trans* nuclear proteins.

**Coarse face, dysostosis multiplex, progressive laryngeal obstruction and severe psychomotor retardation - a new type of autosomal recessive storage disease?** *R. Shenhav<sup>1</sup>, E. Sayag<sup>1</sup>, L. Basel<sup>1</sup>, G. Halpern<sup>1</sup>, A. Rotchild<sup>2</sup>, M. Frydman<sup>3,4</sup>, M. Shohat<sup>1,4</sup>.* 1) Medical Genetics, Rabin Medical Center, Beilinson Campus, Petah Tikva, Israel; 2) Medical Genetics, Hadassah Medical Center, Ein Kerem, Jerusalem, Israel; 3) Medical Genetics, Sheba Medical Center, Tel Hashomer, Israel; 4) Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

We describe an extended consanguineous Israeli Arab family - a brother and sister are married to their first cousins, who are themselves brother and sister. The two couples had together five affected daughters out of nine children. All those affected had similar clinical features: short stature, severe psychomotor retardation, mild microcephaly, coarse and dysmorphic face with depressed nasal bridge, wide nose, full lips, macroglossia, protruding ears, strabismus, and short and broad neck. Other features were small hands and feet with brachydactyly, hyperlaxity of the joints, hirsutism, mild hepatomegaly, but no splenomegaly. There was progressive narrowing of the larynx and trachea, resulting in serious airway compromise, and one girl has already died from this, aged 11 years. Laboratory findings: urinary examination showed normal levels of mucopolysaccharides and oligosaccharides, and no sialic aciduria. Serum arylsulfatase A level and sulfate incorporation by cultured fibroblasts were normal. Skull X-ray revealed a thick calvaria and "J" shaped sella turcica. Skeletal radiological survey showed dysostosis multiplex, platyspondyly, broad ribs, coarsely trabeculated long bones with hypoplastic and irregular epiphyses and broad metaphyses, dysplasia of the hands with hypoplasia and tapering of the distal phalanges, bullet-like proximal phalanges, and acetabular dysplasia. CT and MRI of the skull revealed no specific changes. On rectal biopsy, ultrastructural examination showed no findings specific for mucopolysaccharidosis. Echocardiography was normal. Linkage analysis excluded linkage to the mucopolipidosis 2 gene located on chromosome 4q21-q23. This family represents a previously undescribed type of storage disease that is probably transmitted by autosomal recessive inheritance, and we are currently undertaking linkage analysis.



**Coding region mutations in the MCAD and VLCAD genes may have unforeseeable consequences due to disruption of potential splice enhancer sequences.** *B.S. Andresen<sup>1,2</sup>, K.B. Nielsen<sup>1,2</sup>, T.J. Corydon<sup>1</sup>, L.D. Schroeder<sup>1,2</sup>, N. Gregersen<sup>2</sup>.* 1) Inst Human Genetics, Aarhus Univ, Arhus C, Denmark; 2) . Research Unit for Molecular Medicine, Aarhus Univ. Hospital.

It is becoming clear that coding region mutations do not always exert their effect by simply changing the amino acid sequence of the encoded protein. Instead, the main effect can be a lowering of the amounts of mutant mRNA (mediated by nonsense mediated decay (NMD)) or missplicing of the mRNA. We have monitored the amounts of medium-chain acyl-CoA dehydrogenase (MCAD) mRNA from alleles with >20 different disease-causing mutations using allele specific quantitation in LightCycler based mutation specific assays. All alleles with mutations that generated premature termination codons (PTC)'s upstream of the last exon resulted in low levels of mRNA. Surprisingly, one of the missense mutations (362C>T), located in exon 5, far from both splice junctions, also resulted in low MCAD mRNA amounts. Analysis of patient MCAD cDNA showed that this was caused by complete skipping of exon 5 resulting in a shifted reading frame ending with a PTC in exon 6, thus triggering NMD. Using transfection studies in COS-cells with wild-type and mutant (362C>T) MCAD mini-gene constructs we could reproduce the exon 5 skipping from the mutant construct. Mutagenesis of pos. 362, the neighboring positions, and the suboptimal 3'splice site of intron 4, indicated that exon 5 skipping is probably caused by disruption of an exonic splice enhancer (ESE) sequence. Studies aimed at identifying the relevant splice factor is underway. Similarly, we have identified a potential ESE sequence in exon 12 of the very-long-chain acyl-CoA dehydrogenase (VLCAD) gene, which when disrupted by a missense mutation (1213G>C) leads to exon skipping in patient cells. Studies employing mutagenesis of pos.1213, the neighboring positions and the suboptimal 3'splice site of intron 11 of our VLCAD mini-gene is underway. We conclude that disruption of ESE sequences, which are necessary for correct splicing of exons with a suboptimal 3'splice site, may lead to unpredictable consequences of simple coding region mutations.

**Function-Structure Relationship of Saposin B: An Ex-Vivo Study.** *M. Wang, X. Qi.* Dept. and Program of Human Genetics, Children's hospital, Cincinnati, OH.

Saposin B, a physiologic activator of arylsulfatase A, is essential for the hydrolysis of sulfatide by arylsulfatase A in lysosome. Patients lacking saposin B show variant forms of metachromatic leukodystrophy. In order to investigate the function-structure relationship of saposin B, an ex-vivo system was established. Lissamin-rhodamin labeled sulfatide (LRh-CS) was synthesized as a fluorescent substrate of arylsulfatase A. The characterization of this fluorescent substrate was analyzed by TLC, spectrometer and mass spectroscopy. Liposomes containing LRh-CS and entrapping normal or mutant saposin Bs were used for delivery to cells. The degradation of LRh-CS was activated by external saposin Bs. Degradation was quantified by comparing the fluorescence intensity measured using Laser Screen Microscope. Acriflavin, a lysosomal marker, was used as an internal standard. The staining of acriflavin per sq.mm. cell section was equal in wild type and saposin B mutant cells. Our experiments showed co-localization of LRh-CS and acriflavin in human saposin B mutant cell. In addition, The recombinant saposin B was localized mainly in lysosomes with immune-fluorescence study. By co-loading of recombinant human saposin B, the amount of LRh-CS accumulated in human saposin B mutant cells was significantly reduced by 85%. A substantial accumulation of the loaded substrate LRh-CS was present in granular vesicles in cytoplasm in mouse total saposin (*sap-/sap-*) deficient cells and human saposin B mutant cells. In contrast, this staining pattern was not observed in loaded cells from prosaposin transgenic (*sap-/sap-*) mice and wild type cells. The pattern in these cells was a crescent-shape around the nucleus. Variety of chimeric and mutant saposin Bs was generated to identify the bioactive sites essential for its function.

**Molecular, biochemical, and phenotypic analysis of a male with X-linked dominant Conradi-Hunermann-Happle syndrome (CDPX2).** *J.M. Milunsky<sup>1</sup>, T.A. Maher<sup>1</sup>, A.B. Metzenberg<sup>2</sup>, R.I. Kelley<sup>3</sup>.* 1) Center for Human Genetics and Dept Pediatrics, Boston Univ Sch Medicine, Boston, MA; 2) Dept Biology, California State Univ, Northridge, CA; 3) Kennedy Krieger Inst, Baltimore, MD.

X-linked dominant Conradi-Hunermann-Happle syndrome (CDPX2; MIM 302960) is a rare chondrodysplasia punctata primarily affecting females. CDPX2 is presumed lethal in males, although a few affected males have been reported. CDPX2 is a cholesterol biosynthetic disorder due to 3 $\beta$ -hydroxysteroid-d8,d7-isomerase deficiency caused by mutations in the emopamil binding protein (EBP) gene. A two-year-old Caucasian male was followed from the age of six weeks and noted to have significant developmental delay, hypotonia, seizures, and patchy hypopigmentation. Multiple congenital anomalies included a unilateral cataract, esotropia, crossed renal ectopia, stenotic ear canals, failure to thrive requiring G-tube placement, and microcephaly. Multiple dysmorphic features and ptosis were noted. No skeletal asymmetry or chondrodysplasia punctata were noted on skeletal survey at six weeks and thirteen months. An extensive genetic workup including cholesterol (126-176 mg/dl) and 7-dehydrocholesterol was unrevealing. However, the levels of 8(9)-cholestenol and 8-dehydrocholesterol were mildly increased in plasma which was confirmed in cultured fibroblasts. This prompted molecular analysis of the EBP gene which revealed a novel hemizygous (non-mosaic) mutation in exon 2 (L18P). Expression studies on skin fibroblasts are in progress. Two restriction digests were developed that confirmed this mutation in skin fibroblasts, blood, and buccal cells (all non-mosaic). We determined that the patient's mother (adopted) also has the L18P mutation enabling future prenatal diagnosis. She has normal stature, no asymmetry, no cataracts at this time, and has a patch of hyperpigmentation on her chest best appreciated on Woods lamp exam characteristic of CDPX2. The mild maternal phenotype has been described previously. However, this missense mutation has resulted in a severe phenotype in our surviving male.

**Female carriers of adrenoleukodystrophy show skewed patterns of X inactivation but no favorisation of the mutant allele.** *E.M. Maier<sup>1</sup>, A.C. Muntau<sup>1</sup>, S. Kammerer<sup>2</sup>, A.A. Roscher<sup>1</sup>*. 1) Dr. von Hauner Children's Hospital, Clinical Chemistry and Biochemical Genetics, LMU, Munich, Germany; 2) SEQUENOM Inc., San Diego, CA.

X-linked adrenoleukodystrophy (X-ALD) is the most frequent genetically determined peroxisomal disorder, which is biochemically characterized by an accumulation of very long chain fatty acids (VLCFA) in tissues and body fluids. It is caused by mutations in the *ALD* gene encoding a peroxisomal membrane protein, that is a member of the superfamily of ATP-binding cassette transporters. X-ALD leads to a progressive demyelination of the central nervous system with a wide range of phenotypic expression. Unlike most carriers of X-linked disorders, the majority of X-ALD carriers develop neurological abnormalities at later ages. In the past, this had been explained by skewing of X inactivation favoring the mutant allele.

We analyzed X-chromosome inactivation in peripheral blood cells of carriers and noncarriers from 18 ALD families as well as of healthy controls, using a PCR-based assay at the human androgen receptor locus (HUMARA). Highly skewed patterns (>80:20) were found in 7 of 22 carriers, but were not observed in 7 related noncarriers and 35 unrelated controls. The distribution of X inactivation patterns in X-ALD carriers was clearly different from the bell-shaped normal distribution with predominant random X inactivation demonstrated by both noncarriers and unrelated female controls. The differences were statistically highly significant. Skewing of X inactivation in peripheral blood did not correlate with plasma VLCFA levels. Among the X-ALD carriers with skewed patterns of X inactivation we found association with either favoring the mutant or the wildtype allele. No clear favorisation of the mutant *ALD* allele as the preferentially active one could be delineated. Thus, the results of this study challenge the so far postulated hypothesis of favorisation of the mutant allele. The finding that both favorisation of the mutant or the wildtype allele can be observed in X-ALD carriers might reflect the influence of modifying factors affecting postinactivation selection mechanisms.

**Variation in three SNPs in the calpain-10 gene not implicated in conferring susceptibility to type 2 diabetes in a large Finnish cohort.** *T.E. Fingerlin<sup>1</sup>, M.R. Erdos<sup>2</sup>, R.M. Watanabe<sup>3</sup>, T.T. Valle<sup>4</sup>, J. Tuomilehto<sup>4</sup>, R.N. Bergman<sup>3</sup>, M. Boehnke<sup>1</sup>, F.S. Collins<sup>2</sup> for the FUSION Study Group.* 1) University of Michigan, Ann Arbor, MI; 2) NHGRI, NIH, Bethesda, MD; 3) USC Keck School of Medicine, Los Angeles, CA; 4) National Public Health Institute, Helsinki, Finland.

In recent study of Mexican Americans (MA) Horikawa et al. found that an intronic SNP, UCSNP-43, in the calpain-10 gene showed evidence for linkage and association with type 2 diabetes (T2D), with the common G allele the at-risk allele. Further analyses identified a pair of three-SNP (UCSNP-43, 19, 63) haplotypes that in combination were strongly associated with T2D. Homozygotes for either of the two haplotypes were not at increased risk. We genotyped UCSNP-43, 56, and 63 (UCSNP-56 is in complete linkage disequilibrium with UCSNP-19 and was more easily typed) in 906 Finnish subjects from the FUSION study: 506 index cases with T2D, 179 non-diabetic spouses of index cases or their siblings, and 221 normal glucose tolerant elderly control subjects. We found no significant differences in allele or genotype frequencies between cases and spouses and elderly controls for any of the three SNPs (all p-values > 0.25). We also estimated haplotype frequencies for cases and spouses and elderly controls using the three SNPs. Preliminary results suggest that the high-risk haplotype combination identified by Horikawa et al. is rare in our sample. Within each study group, we compared T2D-related traits (insulin secretion and sensitivity measures, lipid profiles, age-of-onset, and BMI) between subjects with and without the common G allele and among subjects with each of the three genotypes (GG, GA, AA) at UCSNP-43 after adjusting for age and gender. We found no important differences. These results suggest that in this Finnish population 1) UCSNP-43 neither confers susceptibility to T2D nor strongly influences several T2D-related traits and 2) the high-risk haplotype combination identified in the MA population cannot play a substantial role in conferring susceptibility to T2D due to the low frequency of that haplotype combination in Finnish T2D families.

**Linkage disequilibrium in the genes of the renin-angiotensin system; Findings from the Family Blood Pressure**

**Program.** *R.S. Cooper*<sup>1</sup>, *D. Yan*<sup>2</sup>, *X. Zhu*<sup>1</sup>, *A. Luke*<sup>1</sup>, *A. Weder*<sup>3</sup>, *A. Chakravarti*<sup>2</sup>. 1) Preventive Medicine & Epid, Loyola University Chicago, Maywood, IL; 2) McKusick-Nathans Institute of genetic Medicine, Johns Hopkins University, Baltimore, MD; 3) Division of Hypertension, School of Medicine, University of Michigan, Ann Arbor, MI.

Association studies of candidate genes for complex traits like hypertension conducted thus far have relied primarily on information from only one nucleotide polymorphism or an alu element. Variation in the extent of linkage disequilibrium in the gene or region being examined markedly influences the sensitivity and precision of association studies, however, and it has not been adequately described for most candidate genes. In a sample of 415 whites and 477 blacks we estimated intragenic linkage disequilibrium in the 4 genes of the renin-angiotensin system by typing 21 single nucleotide polymorphisms. All but one of the sets of markers were spaced between 1 and 20,000 kb, thus providing resolution at relatively a small scale. Strong linkage disequilibrium existed across all the pairs of markers in whites, with the exception of one polymorphism in the angiotensin I receptor gene. Among blacks there was substantially less linkage in renin, angiotensin-converting enzyme, and the angiotensin receptor, but not angiotensinogen. The average half-length of linkage disequilibrium was about 60 kb in whites and 8 kb in blacks, with the correspondingly greater number of haplotypes among blacks. These findings have implications for fine mapping of candidate genes and suggest that variation between populations of European and African origin in haplotype diversity is characteristic of most genes.

**Genome-wide association study to identify genes related 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>, Y. Nakamura<sup>1,2</sup>, T. Tanaka<sup>1</sup>.* 1) SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Tokyo, Japan; 2) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

To clarify genetic backgrounds in the pathogenesis of acute myocardial infarction (MI), we have launched a genome-wide association study in a Japanese population using 100,000 cSNPs. As a first step, we are now genotyping 94 MI patients and 564 general population by high-throughput multiplex PCR-Invader assay method. We temporarily chose p value of 0.01 to be threshold to select SNPs for further verification by expanding sample size. At present, we have screened 2,609 SNPs and 96% of them did not meet this threshold. When the rest of the SNP loci were re-examined with additional 1,000 MI patients and 1,000 general population, almost all of them did not show statistically significant association. However, one SNP locus showed a tight association, with a  $\chi^2$  value of 21.52 ( $p=0.0000035$ ) and odds ratio of 1.74 (95% confidence interval 1.38-2.21). We are now making a dense SNP map of this locus for linkage disequilibrium mapping, followed by functional analyses of gene products.

**A novel locus for autosomal dominant non-syndromic deafness (DFNA41) maps to chromosome 12q24-qter.** X.Z. Liu<sup>1,2</sup>, S.H. Blanton<sup>1,3</sup>, C.Y. Liang<sup>2</sup>, M.W. Cai<sup>2</sup>, A. Pandya<sup>1</sup>, B. Landa<sup>1</sup>, S. Mummalanni<sup>1</sup>, K.S. Li<sup>2</sup>, L.L. Du<sup>2</sup>, X.N. Qin<sup>2</sup>, Y.F. Liu<sup>2</sup>, W.E. Nance<sup>1</sup>. 1) Dept Human Genetics, VCU, Medical Col Virginia, Richmond, VA; 2) Dept Otolaryngology, West China University of Medical Sciences, Chengdu, China; 3) Dept Pediatrics, University of Virginia, Charlottesville, Virginia.

Hearing impairment is the most common sensory disorder in humans, and genetic factors are a major cause. Approximately 80% of hereditary hearing loss is non-syndromic without associated abnormalities. It is estimated that 15-25% of these cases exhibit an autosomal dominant pattern of transmission. At least 40 autosomal dominant loci have been mapped to date, and of these, 13 have been cloned. We have mapped a novel locus for autosomal dominant non-syndromic hearing loss, DFNA41, to chromosome 12q24-qter in a large multigenerational Chinese family with progressive hearing impairment. Most affected individuals noticed hearing impairment after their teens with subsequent gradual progression from moderate to profound loss. All affected individuals had bilateral sensorineural hearing loss involving all frequencies. There were no obvious vestibular dysfunction and other associated abnormalities. Following a genome wide scan performed by CIDR, a maximum multipoint lod score of 8.97 was obtained at marker D12S343. While multipoint analysis places the DFNA41 locus distal to D12S1609, there was no recombination with other more distal markers to permit identification of a distal flanking marker. Another deafness locus, DFNA25, has been mapped to 12q21-q24. We excluded linkage of the markers surrounding this locus to our family. The DFNA41 interval begins approximately 35 cM distal to DFNA25. Numerous genes are known to map to the region, and among the positional candidate genes, those functionally related to hearing or expressed in the inner ear are being screened for deafness-causing mutations. This work was supported by NIDCD grants DC04530, DC02530, and DC 04282.



**Linkage of autosomal dominant hearing impairment to a locus in 19q13.3-q13.4 and exclusion of BAX, a BCL2-related apoptosis regulator expressed in the cochlea.** *M. Pfister*<sup>1</sup>, *F. Mirghomizadeh*<sup>1,6</sup>, *M. Devoto*<sup>2</sup>, *B. Bardtke*<sup>6</sup>, *J. Oeken*<sup>4</sup>, *S. Koenig*<sup>4</sup>, *E. Vitale*<sup>3</sup>, *A. Riccio*<sup>5</sup>, *A. de Rienzo*<sup>5</sup>, *HP. Zenner*<sup>1</sup>, *N. Blin*<sup>6</sup>. 1) Otolaryngology, University of Tuebingen, Tuebingen, Germany; 2) AI duPont Hospital for Children, Dept. of Research, Wilmington, DE, USA & Department of Oncology, Biology, and Genetics, University of Genoa, Italy; 3) Dept. Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark NJ & CNR Institute of Cybernetics, Naples, Italy; 4) Otolaryngology, University of Leipzig, Leipzig, Germany; 5) Dept. Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark NJ; 6) Institute of Anthropology and Human Genetics, Tuebingen, Germany.

Until now, 32 loci have been identified by linkage analysis of affected families that segregate with non-syndromic and dominantly inherited form of hearing impairment (DFNA). Based on these data, 11 genes for DFNAs were cloned and their majority was characterized. A German family with a non-syndromic progressive hearing impairment transmitted in autosomal dominant modus was linked to 19q13.3-q13.4 by a genome-wide scan. Due to the low lodscore (1.85 at  $q=0.05$ ) for APOC2-locus we extended the fine mapping attempt with further markers in the same chromosomal region. This resulted in significant evidence for linkage to the markers D19S246 and D19S553 (two-point lodscores of 4.05 and 3.55 at  $q=0.0$ ) and a candidate critical region of 14 cM between markers D19S412 and D19S571. This region shows partial overlap with the previously reported DFNA4 critical region. The human gene BAX is orthologous to rodent Bcl2-related apoptosis gene that is temporally expressed in postnatal period of the developing inner ear of the mouse. BAX maps at a distance of no more than 0.73 cM distally to marker D19S553, and thus it appeared a likely candidate in our pedigree. Genomic sequencing of coding regions and exon/intron boundaries resulted in no indication for disease-related mutations, hence, most likely excluding BAX as a candidate gene. However, additional ESTs in the same region remain to be tested.

**Evidence that Connexin 26 35delG does not represent a mutational hotspot.** *C.R. Rothrock<sup>1</sup>, A. Murgia<sup>4</sup>, E. Leonardi<sup>4</sup>, E. Sartorato<sup>5</sup>, L. Bean<sup>1</sup>, R. Fisher<sup>2</sup>, J. Elfenbein<sup>3</sup>, K. Friderici<sup>1</sup>.* 1) Microbiology & Mol. Gen; 2) Peds & Hum. Dev; 3) Audiology and Speech Path., Michigan State University, East Lansing, MI; 4) Dept Pediatrics, Univ. Padua, Padua, Italy; 5) CBMEG, Laboratorio de Genetica Humana, Universidade Estadual de Campinas, Campinas, SP, Brazil.

Non syndromic hearing impairment (NSHI) is the most common form of deafness and presents with no other symptoms or sensory defects. Mutations in the gap junction gene GJB2 account for a high proportion of recessive NSHI. The GJB2 gene encodes connexin 26 that forms plasma membrane channels between cochlear cells. In Caucasian populations a single mutation, 35delG, accounts for most cases of NSHI. This mutation appears to be most prevalent in individuals of Mediterranean European descent, with carrier frequencies estimated as high as one in thirty. Because the 35delG mutation arises from the deletion of one G in a stretch of six guanines, and also because microsatellite markers previously tested show little evidence for linkage disequilibrium, it is possible that this may be a mutational hotspot. However, we believe that 35delG is an old mutation in a chromosomal region of high recombination. To distinguish between an old or recurring mutation, the genetic context of the 35delG mutation was examined. We identified two single nucleotide polymorphisms (SNP) immediately upstream of the first exon of GJB2. PCR-RFLP analysis determined the SNP genotype of 35delG containing chromosomes from various populations, including North America, Italy and Brazil. We find the same relatively rare polymorphism associated with the 35delG mutation in all populations studied. We have also examined microsatellite markers D13S175, which is 80 kb centromeric to GJB2, and D13S1316, which is 80 kb telomeric to GJB2. D13S175 appears to be in strong linkage disequilibrium with 35delG, while D13S1316 shows less linkage disequilibrium. This indicates there has been substantial recombination around the 35delG mutation, especially telomeric to the gene. Our data suggests the 35delG mutation is not a recurring mutation at a mutational hotspot, but is more likely an older mutation that has been maintained in the Caucasian population.

**Development of a laboratory information management system for high throughput SNP genotyping.** *A.A. Chen, K. Embree, J. Bryce, G. Lichtman, A. Mailman, K. Munnely, J.S. Rennich, R. Stanton, T. Yamartino, A. Parker.* Millennium Pharmaceuticals, Cambridge, MA.

The completion of the Human Genome Project has made available to geneticists a vast number of candidate SNPs. Seeking to discover SNPs associated with neuropsychiatric disease, we studied 2251 individuals drawn from a population characterized by an elevated incidence of schizophrenia. In this sample we genotyped a large number of polymorphisms closely spaced in a region known to exhibit linkage to the disease using TaqMan assays. We attempted to configure markers for 78 SNPs, with 92% success; these 72 markers were typed in the study sample with an average of 95% completeness and greater than 99% accuracy. The design and implementation of a comprehensive Laboratory Information Management System (LIMS) is a prerequisite for successful high throughput SNP genotyping on this scale. We have designed and implemented a LIMS permitting us to generate and analyze up to 23,000 SNP genotypes per day.

The LIMS has four fully modular components. The first module is a database system that inventories SNP markers including oligonucleotide sequences, PCR reaction conditions, and physical storage locations. The second module is an allele-calling program that graphically displays data points and allows the user to assign allele calls to the data. A third module directs automated assembly of the PCR reactions on a Tecan Genesis liquid-handling robot. The fourth module is an information management system that inter-connects the other modules, providing barcode label control over disposable labware, and implementing a secondary QC analysis of scored genotypes prior to final storage.

**Integrated genotype/phenotype analysis of complex genetic disorders using the Discovery Manager™ Database.**

*C. Lopez-Correa*<sup>1</sup>, *E. Legius*<sup>2</sup>. 1) Genomica Corporation UK, Ltd Berkhamsted; 2) Center of Human Genetics KULeuven, Belgium.

Now, more than ever, there is an increasing need to handle genetic and population studies due to the overwhelming volume of data and variety of sources. One of the present challenges in genetic research is the study of complex genetic disorders using data from different sources including clinical data, genotype data and mapping data. For those researchers that want to perform genotype/phenotype correlations in complex genetic disorders from multi-disciplinary and multi-site projects this task is even more difficult. Discovery Manager™, a software solution from Genomica, provides an integrated, flexible, easy-to-use database and analysis environment in which genetic, phenotypic, molecular and population-derived information can be stored. This data may be visualized, manipulated and mined with simple and intuitive data management tools specifically designed for genetic and molecular studies. All stored information can be rapidly transformed and exported in the rich formats required for further research and analysis using third party programs such as CRIMAP, MULTIMAP, GENEHUNTER 2.0, LINKAGE, DISEQ, HAPLO, MAPMAKER/SIBS, SAGE/SIBPAL, SIMWALK 2, GAS and SAS. Here we show an example using NF1 microdeletion patients (*Am J Hum Genet.* 2000 ;66(6):1969-74 and *Hum Mutat.* 1999;14(5):387-93). All the genotype and phenotype data from the patients was stored and analyzed in Discovery Manager™. Physical mapping from the deletion region as well as sequence analysis from the sequences involved in the deletion process could also be analyzed using DMTM. Using Discovery Managers analysis tools, the subject population can be stratified based on the severity of the phenotype, and correlation between the particular phenotype and the genotype (markers/ deletion size) can be performed. The sequence analysis tools could also be used to identify new genes located in the deletion region that might be implicated in the phenotype. Aside from this application, Discovery Manager supports a wide range of workflows such as genome-wide linkage scan, association studies and pharmacogenomics.

**Improved design of genetic studies using computer generated data.** *F. Sievers, E. Kelly.* Hitachi Dublin Laboratory, Trinity College, Dublin, Ireland.

Any gene hunting study, be it association or family based, requires an initial design of experiment. This design is usually hampered by uncertainties concerning the dimensioning of the study. For example, how big a stretch of the genome to span, what density of markers, which kind of markers and how many patients or families? Is the total population big enough to support a meaningful analysis or does one have to move to a different population? We propose to use computer generated genetic data to furnish at least approximate answers to these questions. Our population generator traces a set of loci for a whole population over a specified number of generations. We simulate target genes (associated, for example, with diseases or traits) and markers. The number of loci, allele frequencies and recombination ratios between loci can be selected by the designer of the experiment to simulate all possible and impossible scenarios. At different time steps the generated genetic data can be analysed to see if the experiment design is capable of detecting the desired signal (e.g., linkage disequilibrium). We have chosen the Transmission Disequilibrium Test (TDT) as an example to demonstrate the benefits of our technique. A disease gene was initially introduced among microsatellites and SNPs. The population was evolved over 50 generations, at each step guaranteeing random mating but avoiding consanguinity. After a number of generations a signal emerged due to recombinations of the more remote marker loci, confirming the location of the introduced disease gene. As time elapsed an exponential decay of linkage was observed due to recombinations in the vicinity of the disease locus. To assess the accuracy of the TDT results we bootstrapped the population data. Varying the patient sample size was clearly reflected in the size of the error bars observed, thus giving an estimate for the smallest possible population size required to give a reliable signal. We also studied the effect of marker spacing and type of marker (SNP/microsatellite) on the ability to detect linkage.

**An association between an aggrecan gene polymorphism and idiopathic scoliosis.** *T.I. Axenovich<sup>1</sup>, I.V.*

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Idiopathic scoliosis (IS) is defined as a lateral curvature of spine and is a complex inherited disease of unknown etiology. We have performed a segregation analysis of IS on 101 families from Siberian population and found evidence for autosomal major-gene control of the disease with incomplete gender- and age-dependent penetrance. The next step is the mapping of the disease predisposing gene. For that purpose we have used the candidate gene approach, with the aggrecan gene as the candidate gene for IS. Due to the different number of tandem repeats in the alleles of this gene, the aggrecan core proteins are of different length, which may lead to changes in the functional properties of the cartilage. As has been demonstrated, aggrecan gene polymorphism is related to some bone malformations, for example, lumbar disc degeneration. We have performed TDT test on 33 unrelated simplex families (i.e. proband and his/her parents), selected from the sample used in the previous segregation analysis. We have found 9 alleles of aggrecan gene in the sample analyzed, of which three (containing 25, 26 and 27 tandem repeats) were most frequent. We did not detect preferential transmission of any of these alleles to the affected offspring (TDT statistics for an allele vs. all the others varied from 0.0 to 0.71). The global TDT test of marginal heterogeneity did not reveal any association, either. No correlation has been found between the number of tandem repeats and the degree of spine deformation. Thus, either the number of tandem repeat polymorphism of the aggrecan gene is irrelevant to the development of idiopathic scoliosis in the families under consideration, or the effects of this polymorphism are too small to be detected given the sample size chosen.

**Linkage of femoral neck bone mineral density to a quantitative trait locus on chromosome 1p36 by variance component analysis.** *M. Devoto*<sup>1,2</sup>, *C. Specchia*<sup>1,3</sup>, *H-H. Li*<sup>1</sup>, *J. Caminis*<sup>4</sup>, *A. Tenenhouse*<sup>4</sup>, *H. Rodriguez*<sup>5</sup>, *L.D. Spotila*<sup>5</sup>. 1) Dept. Research, AI duPont Hospital for Children, Wilmington, DE; 2) Dept. Oncology, Biology, and Genetics, University of Genoa, Genoa, Italy; 3) Dept. Health Science, University of Genoa, Genoa, Italy; 4) McGill Bone Centre, Montreal, Canada; 5) Dept. Bioscience and Biotechnology, Drexel University, Philadelphia, PA.

Osteoporosis is a common condition characterized by reduced skeletal strength and increased susceptibility to fracture. Eight million Americans over the age of 50 have osteoporosis of the femoral neck. The most important risk factor for osteoporosis is low bone mineral density (BMD), and several epidemiological studies have shown the importance of genetic factors in determining variability of BMD. Our initial genome screen in seven large pedigrees produced suggestive evidence for a candidate region conferring susceptibility to low bone mineral density of the femoral neck located on chromosome 1p36. We have now confirmed and extended this finding by typing nine microsatellite markers spanning a 40 cM interval across our candidate region in a total of 42 Canadian families. Heritability of femoral neck BMD was estimated as  $0.51 \pm 0.13$  in our families, after accounting for the effects of age, sex, BMI, height and weight. Variance component linkage analysis by means of the SOLAR computer program yielded a maximum multipoint lod-score of 3.5 for linkage of femoral neck BMD to a QTL located near marker D1S214, with an associated empirical p-value by simulation analysis of less than 0.00015. More than 50% of the total phenotypic variance was explained by this locus, with no residual additive genetic variance. Our results strongly support the hypothesis that a QTL controlling femoral neck BMD is located on chromosome 1p36, and further analysis of candidate genes in this region is warranted.

**ASSOCIATION BETWEEN TNFR2 AND FAMILIAL BUT NOT SPORADIC RHEUMATOID ARTHRITIS PROVIDES EVIDENCE FOR GENETIC HETEROGENEITY.** *P. Dieude<sup>1</sup>, E. Petit<sup>1</sup>, S. Cailleau-Moindrault<sup>1</sup>, J. Osorio<sup>1</sup>, S. Lasbleiz<sup>2</sup>, B. Prum<sup>1</sup>, T. Bardin<sup>1,2</sup>, F. Cornelis<sup>1,2</sup>.* 1) Laboratoire de recherche Européen pour la Polyarthrite Rhumatoïde, Genopole, for ECRAF, EVRY, FRANCE; 2) Centre Viggo-Petersen, Hôpital Lariboisière, PARIS, FRANCE.

**Background.** Tumor necrosis factor alpha (TNF $\alpha$ ) involved in rheumatoid arthritis (RA) binds TNFR1 and TNFR2 receptors. Genome scans have suggested the TNFR2 locus as a candidate RA locus. A case-control study in a United Kingdom (UK) Caucasian population has shown an association between a TNFR2 genotype (196R/R in exon 6) and familial, but not sporadic RA. **Objective.** To test this association in the French Caucasian population. **Methods.** To test for an association in sporadic RA, 100 families were genotyped for the 196M/R polymorphism and analyzed using the transmission disequilibrium test and the haplotype relative risk (HRR). To test for an association in familial RA, RA index cases from affected sib pair (ASP) families (n = 100) were genotyped for 196M/R. Linkage analysis was performed with 3 TNFR2 microsatellite markers. **Results.** The TNFR2 196R/R genotype was not associated with sporadic RA (odds ratio [OR]: 0.59, P= 0.72), but with familial RA (OR: 4, P= 0.026). The association was most marked in the context of TNFR2 twin-like RA sibs (affected sibs sharing both TNFR2 haplotype) (OR: 9.73, P= 0.0017). Linkage analysis was consistent with the association, the subgroup of families with 196R/R ASP index cases provided most of the TNFR2 linkage evidence. **Conclusion.** This study represents the first replication of the involvement of TNFR2 in RA genetic heterogeneity. Our data refine the initial hypothesis, to suggest that a TNFR2 recessive factor, in linkage disequilibrium with the 196R allele, plays a major role in a subset of families with multiple RA cases.



**Genome Scan for Hand Osteoarthritis: The Framingham Study.** *S. Demissie*<sup>1</sup>, *L.A. Cupples*<sup>1</sup>, *R.H. Myers*<sup>2</sup>, *P. Aliabadi*<sup>2</sup>, *D. Felson*<sup>2</sup>. 1) School of Public Health, Boston University, Boston, MA; 2) School of Medicine, Boston University, Boston, MA.

Osteoarthritis (OA) is the most common form of arthritis and is among the leading causes of disability. As part of the complex etiology of this disease, there is an increased recognition that a genetic component plays an important role, 20-50% heritability. Our objective was to search for markers that are linked to hand OA using radiographic data in the Framingham Heart Study. The sample included 684 original cohort members (parents) and 793 offspring in 296 pedigrees. The average age at which the hand radiographs were taken was 62 years for the original cohort and 54 years for the offspring. Radiographs were read by one expert reader, and OA features evaluated included Kellgren and Lawrence (K/L) grade, osteophytes (OST), and joint space narrowing (JSN) scores (0-3 scale). Four quantitative hand OA phenotypes were computed from these measurements: sum of KL scores across hand joints, sum of OST scores, sum of JSN scores, and proportion of affected joints. Prior to linkage analysis these phenotypes were adjusted for age and sex using a linear regression analysis from which standardized residuals were computed. The variance component model (SOLAR) was then applied to the normalized scores of the residuals. Linkage was considered significant if the LOD score exceeded 3.0 and suggestive if it exceeded 1.5. Fifty percent of the original cohort members and 30% of their offspring had at least one affected joint defined as K/L grade<sup>3</sup>. Heritability of hand OA ranged between 28% ± 6% for the proportion of joints affected with OA to 34% ± 6% for the sum of KL grades. Eight chromosomal regions indicated suggestive linkage for at least one of the four phenotypes. For these chromosomal regions LOD scores were highest for JSN, with a LOD=2.96 on 1p at D1S1665. Chromosomes 7, 9, 13, and 19 indicated consistent LOD score elevation for multiple phenotypes. The results of this study suggest that there are several chromosomes that may harbor osteoarthritis susceptibility genes. Further investigation of these regions using larger studies and finer maps will be important to confirm linkage.

**Association of Fcγ receptor gene polymorphisms in the Japanese patients with systemic lupus erythematosus: Independent contributions from *FCGR2B* and *FCGR3A*.** C. Kyogoku<sup>1</sup>, H.M. Dijkstra<sup>2,3</sup>, N. Tsuchiya<sup>1</sup>, Y. Hatta<sup>1</sup>, H. Kato<sup>1</sup>, A. Yamaguchi<sup>1</sup>, T. Fukazawa<sup>4</sup>, M.D. Jansen<sup>3</sup>, H. Hashimoto<sup>4</sup>, J.G.J. van de Winkel<sup>3</sup>, C.G.M. Kallenberg<sup>2</sup>, K. Tokunaga<sup>1</sup>. 1) The University of Tokyo, Japan; 2) University Hospital Groningen, Netherlands; 3) University Medical Center Utrecht, Netherlands; 4) Juntendo University, Japan.

Human low affinity Fcγ receptors (FcγRs) constitute a clustered gene family located on chromosome 1q23, that consists of FcγRIIA, IIB, IIC, IIIA and IIIB genes. FcγRIIB is unique in its ability to transmit the inhibitory signals through ITIM in its cytoplasmic domain, and recent animal studies demonstrated the role of FcγRIIB in the regulation of autoimmunity. The association of genetic variants of FcγRIIA, IIIA and IIIB with systemic lupus erythematosus (SLE) has been extensively studied, but the results have been inconsistent in various populations. To examine the possibility that another susceptibility gene of primary significance exists within the Fcγ receptor loci, we screened for the polymorphisms of human *FCGR2B* gene. A single nucleotide polymorphism, c.695T>C, was detected, presenting two alleles coding for Ile (232I) and Thr (232T) within the transmembrane domain. The case-control association study using 193 Japanese patients with SLE and 303 healthy individuals demonstrated that 232T/T genotype was significantly increased in the patients (10.9%) compared with the healthy individuals (5.3%,  $c^2=5.6$ ,  $P=0.018$ ). Genotyping was also carried out for *FCGR2A*-131H/R, *FCGR3A*-176F/V and *FCGR3B*-NA1/2 polymorphisms, among which *FCGR3A*-176F/F showed significant association ( $c^2=5.8$ ,  $P=0.016$ ). The two-locus analyses revealed that, while the association of 3A and 2B was independent, the previously reported association of 3B was considered to derive from strong linkage disequilibrium with 2B. In conclusion, our present findings strongly suggested that *FCGR2B* and 3A independently contribute to the genetic susceptibility to SLE in the Japanese population. The role of FcγR region genes for the genetic background of SLE appears to be highly complicated, with multiple genes associated with different aspects of the disease in different populations.

**Evidence for a novel locus for human malignant infantile osteopetrosis on chromosome 6q21.** A. Ramirez<sup>1</sup>, J. Faupel<sup>1</sup>, C. Stoeckle<sup>1</sup>, C. Hasan<sup>2</sup>, U. Bode<sup>2</sup>, P. Propping<sup>1</sup>, C. Kubisch<sup>1</sup>. 1) Institute of Human Genetics, University of Bonn, Germany; 2) Department of Pediatrics, University of Bonn, Germany.

Malignant infantile osteopetrosis (OMIM 259700) is a rare autosomal recessive disease. The clinical findings including osteosclerosis, hepatosplenomegaly and pancytopenia normally become apparent during the first months of life. The disease is thought to be due to an inadequate bone resorption caused by osteoclast dysfunction. Mutations in the human subunit  $\alpha 3$  of the  $H^+$ -ATPase and the voltage-gated chloride channel CLCN7 are known to cause malignant infantile osteopetrosis by impairing osteoclast function. In a small inbred family with malignant infantile osteopetrosis, no mutation in the  $\alpha 3$  subunit of the  $H^+$ -ATPase nor in CLCN7 were found. To identify a novel osteopetrosis locus and to try to unravel the causative gene defect in this family, a candidate locus approach was pursued by homozygosity mapping. We excluded homozygosity for regions harboring several candidate genes which cause severe osteopetrosis in mice, among them c-FOS on 14q24, c-SRC on 20q11, OPGL on 13q14, and CSF-1 on 1p13. We then analyzed a region on human chromosome 6q21 syntenic to mouse chromosome 10 where the causative gene for the osteopetrotic mouse "grey-lethal" has been mapped. The murine critical locus is flanked by Fyn and Ros1, yet, the responsible gene has remained elusive. We detected homozygosity in a region spanning at least 15cM between microsatellite markers D6S1717 and D6S287. In this region several candidate genes, like Fyn-related-kinase (FRK), BET3, and MARCKS are located. We are currently conducting systematic mutation screening for several candidate genes. In summary, we provide evidence for a novel locus for human malignant infantile osteopetrosis on chromosome 6q21 probably representing the human ortholog of the murine "grey-lethal" locus.

**A new syndrome of hereditary sensory and cerebellar ataxia with muscle atrophy, SCA18: linkage to chromosome 7q31-7q32.** Z. Brkanac<sup>1</sup>, M. Fernandez<sup>4</sup>, M. Matsushita<sup>2</sup>, H. Lipe<sup>5</sup>, T.D. Bird<sup>2,3,5</sup>, W.H. Raskind<sup>1,2,6</sup>. 1) Depts of Psychiatry; 2) Medicine and; 3) Neurology, University of Washington, Seattle, WA; 4) Dept of Neurology, Ohio State University, Columbus OH; 5) GRECC and; 6) MIRECC, VA PSHCS, Seattle, WA.

The autosomal dominant (AD) spinocerebellar ataxias (SCAs) are a heterogeneous group of disorders characterized by variable clinical, electrophysiological, imaging and neuropathological profiles. This clinical heterogeneity is paralleled by molecular genetics studies that have so far implicated 13 loci in the etiology of the disease; 7 genes have been identified. There is a substantial clinical overlap between SCAs, and often they cannot be differentiated on the basis of clinical or neuro-imaging studies. We have identified a four generation American family of Irish ancestry with a unique neurological disorder displaying an AD pattern of inheritance. The proband presented with gait ataxia. Onset of symptoms is usually in adolescence, progression is slow and life span normal. There was variable expressivity of symptoms and severity, including ataxia, sensory loss, pyramidal tract signs and proximal weakness. EMG and NCV studies were consistent with sensory axonal neuropathy. Muscle biopsy revealed neurogenic atrophy and brain MRI has shown mild cerebellar atrophy. To identify the responsible locus we pursued a whole genome linkage analysis with ABI PRISM Linkage Mapping Set, Version 2. Two-point linkage was performed with the LINKAGE-MLINK program. After analyzing 114 markers, linkage to D7S486 was identified with a two point LOD score of 4.79 at Q=0.00. Evaluation of additional markers in the region provided a maximum LOD score of 6.36 at Q=0.00 for marker D7S2554. Haplotype analysis delimited an approximately 14 cM region between markers D7S2418 and D7S1804 containing the gene for this novel SCA. The locus was assigned the symbol SCA18 by the Genome Nomenclature Committee. Our latest query of the NCBI database revealed 52 genes mapped to the region. Interestingly, PMPCB, a gene which was identified as a frataxin protein partner and would be an excellent candidate gene, lies 10 cM proximal to the critical region.

**Genetic analysis of an Italian family with dominant focal dystonia adds further evidence for genetic splitting .**

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Primary Torsion Dystonia (PTD) is a neurological disorder characterized by involuntary twisting movements and abnormal posture, in absence of any recognizable secondary cause. Most cases are sporadic, but several PTD families have been reported with autosomal dominant inheritance, reduced penetrance, and variable expression. Four PTD loci (DYT1, DYT6, DYT7 and DYT13) have been mapped, DYT1 being the only gene cloned so far. A unique mutation (GAGdel) in the DYT1 gene is responsible for most cases of early limb-onset generalised dystonia, usually with sparing of the cranial-cervical region. DYT6, DYT7 and DYT13, on chromosomes 8p, 18p and 1p36, have been mapped in single PTD families with adult onset and cranial-cervical involvement. Linkage analysis of the DYT6, DYT7 and DYT13 regions was carried out in a three generation family (15 members) from Southern Italy with 6 patients affected by purely focal dystonia. Age of onset was between 20 and 50 years. Furthermore, the DYT1 gene on 9q34 was screened for the GAGdel mutation. In this family, linkage of dystonia with all known PTD loci was excluded, and the DYT1 mutation was not detected. These data suggest that, in this family, dystonia is linked to a novel unassigned gene, and confirm the genetic heterogeneity of adult-onset PTD. The small size of this kindred does not allow a genome-wide search. The clinical features are clearly distinct from those observed in the families linked to the excluded PTD loci. A detailed evaluation of the phenotype in newly recognized PTD families is crucial to identify clinically homogeneous families to be pooled together, in order to assign novel PTD loci. The present study supports that pure focal dystonia with variably affected body parts among family members is a distinct clinical and genetic disorder.

**5-HT6 receptor gene polymorphism and Parkinson's disease.** *G. Annesi<sup>1</sup>, I.C. Ciro Candiano<sup>1</sup>, P. Serra<sup>1</sup>, A.A. Pasqua<sup>1</sup>, F. Annesi<sup>1</sup>, C. Tomaino<sup>1</sup>, P. Spadafora<sup>1</sup>, S. Carrideo<sup>1</sup>, D. Civitelli<sup>1</sup>, E.V. De Marco<sup>1</sup>, L. Mangone<sup>2</sup>, G. Nicoletti<sup>1</sup>, D. Messina<sup>1</sup>, A. Quattrone<sup>1,2</sup>.* 1) Inst Exp Med & Biotech, National Research Council, Cosenza, Italy; 2) Inst Neurol, Dept Med Sci, Univ Catanzaro, Italy.

Parkinson's disease (PD) is neurodegenerative disease characterized by a triad of rigidity, resting tremor and bradykinesia. These symptoms result predominantly from the dysfunction and loss of nigrostriatal dopaminergic neurons. Damage to dopaminergic nigrostriatal neurons in the unifying neuropathology and neurochemical feature of the disease. Studies have reported reduced 5-HT concentration in patients with PD, and evidence suggests that serotonin (5-HT) may modulate dopaminergic activity in the striatum. The effects of 5-HT in the brain are mediated through several receptor subtypes. 5-HT6 gene maps to human chromosome region 1p35-36 and encodes for a receptor protein that positively links to adenylyl cyclase. In this study we conducted a genetic association analysis to verify whether the allelic variant C267T of the human 5-HT6 gene contribute to the risk of developing PD. To test this hypothesis, we studied the C267T polymorphism in 243 patients with PD (142 men and 101 women; mean age  $66.7 \pm 8.8$ ) and 234 healthy control subjects (117 men and 117 women; mean age  $68.0 \pm 15.9$ ) all coming from the same geographical area. Both populations were in Hardy-Weinberg equilibrium. Allele and genotype distribution were significantly different between patients and controls. Patients carried the C allele less frequently than controls and subjects who were homozygous (CC) or heterozygous (CT) for the C267T polymorphism were at reduced risk of PD (OR=0.60; CI= 0.40-0.89;  $p=0.013$ ). The functional significance, if any, of this polymorphism is unknown and this association, although interesting, needs to be replicated.

**Systematic Genome Scan of Alcoholism using Genetic Linkage Analysis in Multiplex British Families supports the presence of susceptibility loci on chromosome 1p and 16p.** *I. Guerrini<sup>1</sup>, C.C.H. Cook<sup>3</sup>, W. Kest<sup>1</sup>, A. Devitgh<sup>1</sup>, A. McQuillin<sup>1</sup>, G. Kalsi<sup>1</sup>, D. Curtis<sup>2</sup>, H.M.D. Gurling<sup>1</sup>.* 1) Molecular Psychiatry Laboratory, Windeyer Institute for Medical Science, Department of Psychiatry and Behavioural Sciences, Royal Free and University College London Medical University of London, 46 Cleveland Street, London W1P 7PN; 2) Joint Academic Department of Psychological Medicine, St Bartholomew's and Royal London School of Medicine and Dentistry, 3rd Floor Alexander Wing, Turner Street, London E1 1BB; 3) Kent Institute of Medicine and Health Science, University of Kent at Canterbury, Canterbury, Kent CT2 7PD.

Previous genome-wide scan studies have reported suggestive evidences of linkage to alcoholism on chromosome 11p, near the tyrosine hydroxylase and the DRD4 loci, on chromosome 4, in two loci, near the locus for the b1-GABA receptor unit and near the ADH gene cluster (Long et al., 1998) and also on chromosomes 1p, 2q, 4p, 7q and 16p (Reich et al., 1998). We carried out a genome scan on medium to large sized British families, densely affected by alcoholism, by using a set of 360 polymorphic microsatellite markers. Linkage analysis was performed out using model-free and a model based lod scores methodologies. Linkage analyses with two point and multipoint analyses have shown positive lod scores on chromosome 1p at D1S1588 (MLOD: 1.8), D1S3723 (MLOD: 1.1) D1S1591 (MLOD: 0.9), D1S532 (MLOD: 1.23), D1S1675 (MLOD: 1.23) and at the locus D16S2622 on chromosome 16p (MLOD: 1.16). These results are supportive of linkage at two regions previously implicated by the USA COGA study on chromosome 1p and on chromosome 16p. Long JC, et al. (1998), *Am J Med Genet*, 81, 216-21; Reich T., Edenberg HJ et al. (1998), *Am. J. Med. Gen.*, 81, 207-215. Funded by the Clinical Research and Development Committee of University College London Medical School, the Sir Samuel Scott of Yew's Trust and the Neuroscience Research Charitable Trust.

**Clinical and genetic study of a family with autosomal recessive Charcot-Marie-Tooth (CMT2AR) from southern Italy mapping to 1q21.2-21.3.** A.L. Gabriele<sup>1</sup>, M. Muglia<sup>1</sup>, A. Patitucci<sup>1</sup>, A. Magariello<sup>1</sup>, R. Mazzei<sup>1</sup>, F.L. Conforti<sup>1</sup>, L. Mangone<sup>2</sup>, R. Nistico<sup>2</sup>, P. Valentino<sup>2</sup>, A. Quattrone<sup>2,1</sup>. 1) Institute of Experimental Medicine and Biotechnology, CNR, Cosenza-Italy; 2) Institute of Neurology, School of Medicine, Catanzaro-Italy.

Charcot-Marie-Tooth (CMT) disease is the most common inherited motor and sensory neuropathy. The axonal form of the disease is designated as CMT type 2 (CMT2) and has been divided into autosomal dominant CMT2 AD and autosomal recessive forms CMT2 AR. Five loci are implicated in autosomal dominant CMT2 and have been mapped on 1p35-p36 (CMT2A), 3q13-q22 (CMT2B), 7p14 (CMT2D) and most recently on 8p21 (CMT2E). A fifth CMT2 type (CMT2C) has not been linked to any known locus. Only two genes responsible for the CMT2A and the CMT2E have been identified as the Microtubule Motor KIF1Bb and neurofilament light (NFL) respectively. Until now, two loci have been associated with the autosomal recessive axonal form: the first has been mapped on chromosome 1q21.2-21.3 in a consanguineous Moroccan family, the second locus has been identified in a Costa Rican family on chromosome 19q13.3. So far only one family has been reported to be linked to the chromosome 1q21.2-21.3, making it difficult to compare clinical features and severity of the disease. In this study we report a pedigree from southern Italy with autosomal recessive CMT2. By analysis of the family we detected linkage to the 1q21.2-21.3. Our data likely locate the CMT2AR gene in a region of approximately 1.7cM between markers D1S303 and D1S506, confirming the previous report that the CMT2AR is genetically linked to the 1p21.2-21.3 region.



**Results of a high resolution genome screen in 443 Alzheimer's disease families: the NIMH Genetics Initiative.** *D. Blacker*<sup>1</sup>, *L. Bertram*<sup>1</sup>, *A.J. Saunders*<sup>1</sup>, *T.J. Moscarillo*<sup>1</sup>, *M.S. Albert*<sup>1</sup>, *H. Wiener*<sup>2</sup>, *R. Perry*<sup>2</sup>, *R.C.P. Go*<sup>2</sup>, *A. Mahoney*<sup>3</sup>, *T. Beaty*<sup>3</sup>, *M.D. Fallin*<sup>3</sup>, *S.S. Bassett*<sup>3</sup>, *E.W. Pugh*<sup>4</sup>, *K.F. Doheny*<sup>4</sup>, *R.E. Tanzi*<sup>1</sup>. 1) Massachusetts General Hospital/Harvard Medical School, Boston, MA; 2) University of Alabama School of Public Health, Birmingham, AL; 3) Johns Hopkins University Medical School/School of Public Health, Baltimore, MD; 4) Center for Inherited Disease Research, Baltimore, MD.

A 9 to 10 centimorgan genome screen of 443 affected sibpairs and other mostly small families with Alzheimer's disease from the National Institute of Mental Health Genetics Initiative sample (n = 1453: 700 affected, 419 unaffected, and 34 with phenotype unknown) was performed at the Center for Inherited Disease Research. Families in which any sampled affected onsetted prior to age 50 were excluded: the mean age of onset in this sample is 72.5 years (s.d. = 7.7), typical for late-onset Alzheimer's disease. The resulting genotypes and up-to-date clinical information--the highest resolution screen of the largest sample collected to date for this disease--were subjected to parametric two-point lod score analyses in LINKAGE, non-parametric two-point identity by descent analyses in Sibpal, and non-parametric multipoint analyses in GENEHUNTER, with additional efforts at localization using the novel multipoint program Genefinder. These analyses were performed for the total sample, and then separately for the late-onset (all sampled affecteds onsetting at or beyond age 65) and the remaining early/mixed strata. There were two peaks that gave strong evidence of genetic linkage (including chromosome 19q13 [the location of APOE], with ZLR = 3.8 in GENEHUNTER), and five additional areas with more modest evidence for linkage (ZLR > 2.5). Each of these peaks was observed across the various methods, and included evidence for linkage at several adjacent markers. Additional work is underway to follow-up on these peaks with flanking markers, and to test promising candidate genes within these regions.

**The A80G polymorphism in the reduced folate carrier (RFC-1) gene as risk factor for NTDs.** *P. De Marco*<sup>1</sup>, *M.G. Calevo*<sup>2</sup>, *L. Arata*<sup>1</sup>, *A. Moroni*<sup>1</sup>, *E. Merello*<sup>1</sup>, *L. Andreussi*<sup>1</sup>, *A. Cama*<sup>1</sup>, *R. Finnell*<sup>3</sup>, *V. Capra*<sup>1</sup>. 1) Lab.Servizio di Neurochirurgia, Istituto Scientifico G.Gaslini, Genova, Genova, Italy; 2) Servizio di Epidemiologia e Biostatistica, Istituto G. Gaslini, Genova, Italy; 3) Center for Human Molecular Genetics, Nebraska Medical Center, Omaha, NE.

Moderate hyperhomocysteinemia in women has been associated with an increased risk of pregnancies affected by neural tube defects (NTDs). Folate supplementation may normalize homocysteine metabolism and decrease NTD risk. The C677T polymorphism of the MTHFR enzyme is an important genetic risk factor. Furthermore, compromise of internalization of folate may be involved in pathogenesis of NTD. Folate transport system is mediated by a low affinity and high capacity membrane protein encoded by the newly discovered RFC-1 (reduced-folate carrier) gene. A common polymorphism, A80G, in exon 2 of RFC-1 gene could be an additional risk factor, since that doubly homozygous 80GG/677TT subjects have moderate increase in homocysteine levels. In a case-control study, we examined the genotype frequencies of A80G polymorphism in the Italian population and evaluated its impact on NTD individuals and their relatives. By means of restriction enzymatic analysis, we determined that the frequency of the G allele was 0.46 among control individual suggesting that the A80G mutation is a common polymorphism, at least among Italian population. Significantly higher frequencies were found among NTD cases (0.57), mothers (0.59) and fathers (0.50). OR and 95% CI for heterozygous 80AG and homozygous 80GG NTD cases were 1.84 (0.97-3.51;  $P < 0.045$ ) and 2.43 (1.18-5.06;  $P < 0.009$ ), respectively. A similar risk was found for homozygous mothers: OR=2.83 (0.93-8.83;  $P < 0.04$ ). The odds ratio were also calculated for heterozygous A80G genotype of the mothers and for heterozygous and homozygous genotype of the fathers, but the results were not statistically significant. These results demonstrate that the RFC-1 80G polymorphism may play a role in NTD risk, at least in the Italian population. Further studies should evaluate of the level of risk conferred by interaction between the A80G mutation and other polymorphisms, especially the C677T of the MTHFR gene.

**Linkage and candidate gene analysis of Idiopathic Epilepsies.** *M.E.S. Bailey<sup>1</sup>, S.A. Carter<sup>1</sup>, E.S. Tobias<sup>2</sup>, P. Kwan<sup>3</sup>, R. Sutton<sup>1</sup>, G.J. Sills<sup>3</sup>, M.J. Brodie<sup>3</sup>, K.J. Johnson<sup>1</sup>.* 1) Div Molecular Genetics, IBLs, Univ Glasgow, Glasgow, U.K; 2) Dept Medical Genetics, Yorkhill Hosp., Glasgow, U.K; 3) Epilepsy Unit, Univ. Dept Medicine and Therapeutics, Western Infirmary, Glasgow, U.K.

The idiopathic epilepsies (IE) are a complex set of common disorders of the brain characterised by the occurrence of unprovoked seizures of unknown aetiology as the main or only symptom. In familial cases, a range of seizure phenotypes may co-exist in different family members and within individuals. It is thought that major genes underlie aspects of seizure susceptibility, while modifier loci of varying effect contribute to seizure type specificity and severity. Recently, progress has been made in mapping and identifying genes involved in IEs, particularly the most clearly familial forms, all of which have turned out to be ion channels, making these the current candidate genes of choice. One recently described syndrome, GEFS+, is caused in a few families by mutations in genes encoding subunits of neuronal sodium channels, *SCN1A*, *SCN2A* and *SCN1B*. Other phenotype combinations are caused by mutations in members of the calcium channel and GABA<sub>A</sub> receptor subunit gene families. Another common form IE, juvenile myoclonic epilepsy (JME), can exist either alone, or with other IEs in the syndrome idiopathic generalised epilepsy (IGE). Recent genome scans of JME and IGE families have implicated several novel chromosomal regions. We have collected both families with IE phenotypes and JME triads with full clinical investigation. In familial cases, we have conducted linkage analyses using markers spanning chromosomal regions harbouring known IE loci. Results to date have shown evidence for lack of linkage across large regions of these chromosomes. We have found a significantly positive score in non-parametric linkage analysis in a GEFS+ family in a novel region of chr.2p. JME patients and the affected individuals from the familial cases have been screened by SSCP and dHPLC for variant mobility fragments in *SCN1A*, *SCN1B*, *CACNB4*, *GABRG2* and several other candidate genes. No variants segregating consistently with affected status have been observed. Candidate gene mutation and association studies are ongoing.

**Clinical and genetic heterogeneity in Joubert syndrome: exclusion of loci on 9q and 17p.** A. Badhwar<sup>1,2,6</sup>, E. Andermann<sup>1,2</sup>, F. Andermann<sup>1,3</sup>, E. Uyama<sup>4</sup>, J. Kamei<sup>5</sup>, S. Bourgoin<sup>6</sup>, M. Labuda<sup>6</sup>, B. Rosenblatt<sup>1,3</sup>, M. Shevell<sup>1,3</sup>, A. Lortie<sup>7</sup>, L. Carmant<sup>7</sup>, M. Pandolfo<sup>1,6</sup>. 1) Depts. of Neurology and Neurosurgery; 2) Human Genetics and; 3) Pediatrics, McGill University, Montreal, Canada; 4) Dept. of Neurology, Kumamoto University School of Medicine, Japan; 5) Dept. of Neurology, Iwate Medical College, Japan; 6) CRLCS, Notre Dame Hospital, Montreal, Canada; 7) Dept. of Neurology, St. Justine Hospital, Montreal, Canada.

Four French-Canadian siblings with agenesis of the cerebellar vermis, episodic alternating hyperpnea and apnea, developmental delay, ataxia, hypertonia, and oculomotor abnormalities were described by Joubert et al. (Neurology 1969;19:813-825). Subsequently, Joubert syndrome (JS) has been recognized as a common autosomal recessive disorder of brain development with variable phenotype (Curr Opin Pediatr 2000;12:523-528). To date, the molecular basis of JS is unknown. Linkage to chromosome 9q34.3 was reported in one consanguineous Arabian-Iranian family (Am J Hum Genet 1999;65:1666-1671). More recently, the description of a patient with the JS phenotype and an unusual Smith-Magenis syndrome (SMS) deletion (del 17p11.2-12) suggested the presence of a JS gene in close proximity to the SMS locus (Am J Med Genet 2000;95:467-72). In the present study, we recruited ten JS families, including the original sibship described by Joubert et al. Seven of these families were of French-Canadian origin, and three of Japanese descent. Significant clinical heterogeneity was found within and among the JS sibships. Genealogical study of the French-Canadian families resulted in a pedigree of 425 people spanning 11 generations, and tracing back to a founder who immigrated to Quebec, Canada from the province of Perche, France in 1634. We genotyped nine families using polymorphic markers (D9S164, D9S1818, D9S114, D9S1826, D9S158, and D9S1838) on the 9q34.3 locus and markers that define the possible JS locus on 17p11.2-12. Haplotype analysis in these families showed no evidence of homozygosity relevant with linkage to either the 9q or 17p loci, suggesting genetic heterogeneity in JS. A genome-wide scan is underway.

***Mvb1* Suppresses Neurodegeneration in the *vibrator* Mouse.** J.A. Floyd<sup>1</sup>, D. Concepcion<sup>2</sup>, E.J. Ward<sup>2</sup>, B.A. Hamilton<sup>1</sup>,  
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*Vibrator* mice have a five-fold decrease in *phosphatidylinositol transfer protein a (PITPa)* gene expression which is caused by an insertion of an endogenous retrovirus into intron 4. The resulting phenotype of the mouse is an early-onset tremor with decreased longevity and neurodegeneration in the brainstem and spinal cord. The CAST-Ei allele of the gene *Modifier of vibrator1 (Mvb1)* was found to suppress these phenotypes in the *vibrator* mice. Specifically, life span of the mice is increased, neurocytology is improved, and *PITPa* expression is increased two-fold. This locus has been mapped to chromosome 19 through 10,000 meioses. The *Mvb1* interval contains nine genes, three of which are expressed in tissues that show *Mvb1* effects on *PITPa* RNA levels and are polymorphic between the two strains. As *Mvb1* affects *PITPa* expression in fibroblasts cultured from *vibrator* mice, *Mvb1* will be identified by *in vitro* complementation.

**Association studies of multiple candidate genes for Parkinson's disease by using single nucleotide**

**polymorphisms.** *Y. Momose<sup>1,2</sup>, M. Murata<sup>2</sup>, K. Kobayashi<sup>1</sup>, M. Tachikawa<sup>1</sup>, N. Hattori<sup>3</sup>, M. Yamamoto<sup>4</sup>, I.*

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Parkinson's disease (PD) is a complex genetic disorder, with multiple genetic and environmental factors influencing disease risk. Although several causal genes of familial parkinsonisms have recently been identified, certain genetic factors associated with pathogenesis of idiopathic PD have not yet been clarified. On the other hand, clinical course and effects or side effects to antiparkinsonian drugs are different between each patient. To identify susceptibility genes for PD and to establish tailor-made medicine for PD, we started association studies by using single nucleotide polymorphisms (SNPs) in candidate genes. 236 patients with PD and 249 normal controls were examined in this study. We studied 20 SNPs of 18 candidate genes for association with PD, namely, (1) dopamine-related genes: AADC, COMT, DBH, DRD3, DRD5, DAT; (2) nerve growth factor-related genes: BDNF, CNTF, NGFB, NTRK1; (3) genes for metabolism or transportation of toxins: CYP1A2, CYP2E1, CYP2C9, PON1, MTHFR, DIA4, ABCB1; (4) genes of familial PD; UCH-L1. We found that homozygosity for the V66M polymorphism of the BDNF(brain derived neurotrophic factor) gene occurs more frequently in PD patients than in unaffected controls ( $c2=5.59$ ,  $p=0.018$ ). In addition, we confirmed that homozygosity for the S18Y polymorphism of the UCH-L1(ubiquitin carboxy-terminal hydrolase L1) gene are less frequent ( $c2=5.87$ ,  $p=0.015$ ). For the tailor-made medicine for PD, we further examined the association between each SNP and susceptibility to subsequent levodopa-associated adverse effects such as dyskinesia, wearing-off type motor fluctuation, and neuroleptic malignant syndrome. However, we saw no significant associations. Our results provide genetic evidence supporting a role for BDNF in the pathogenesis.

Program Nr: 1857 from the 2001 ASHG Annual Meeting

**The amyloid precursor protein locus and very-late-onset Alzheimer's disease.** *J.M. Olson, K.A.B. Goddard, D. Dudek.* Epid/Biostat, MetroHlth Med Ctr, Case Western Reserve Univ, Cleveland, OH.

Although mutations in the amyloid-beta precursor protein (APP) gene are known to confer high risk of Alzheimer's disease (AD) to a small percentage of early-onset families, convincing evidence of a major role for the APP locus in late-onset AD has not been forthcoming. Using data collected through the NIMH AD Genetics Initiative, we analyzed the chromosome 21 genome scan data using the covariate-based methods of affected-sib-pair (ASP) linkage analysis described in Goddard et al. [AJHG 68:1197-1206, 2001] and now available in S.A.G.E. By including covariates in the linkage model, one allows for possible locus heterogeneity due to the covariate. Using the number of E4 alleles in the ASP as a covariate, we confirmed an earlier finding using these data that ASPs lacking E4 alleles show some increased evidence for linkage (lod score = 1.13 vs baseline lod score = .02). More importantly, we obtained strong evidence for linkage when age-at-onset (lod=2.27, p=.0032), age-at-exam/death (lod=5.54, p=.000002) or age-at-onset plus disease duration (lod=5.63, p=.000006) were included as covariates in the linkage analysis. These results support a hypothesis that APP mutations are involved in familial AD in the most elderly patients (age-at-exam/death > ~88). Removal of potential outliers does not substantially change the results. These results also highlight the usefulness of covariate-based model-free linkage methods in complex human disease.

**A large family with pure autosomal dominant hereditary spastic paraplegia from southern Italy mapping to chromosome 14q11.2-q24.3.** *M. Muglia*<sup>1</sup>, *A. Magariello*<sup>1</sup>, *G. Nicoletti*<sup>1</sup>, *A. Patitucci*<sup>1</sup>, *A.L. Gabriele*<sup>1</sup>, *F.L. Conforti*<sup>1</sup>, *R. Mazzei*<sup>1</sup>, *M. Caracciolo*<sup>1</sup>, *G. Casari*<sup>2</sup>, *B. Ardito*<sup>3</sup>, *M. Lastilla*<sup>3</sup>, *L. Mangone*<sup>4</sup>, *A. Gambardella*<sup>1,4</sup>, *A. Quattrone*<sup>1,4</sup>. 1) IMSEB-CNR, Mangone Cosenza, Italy; 2) Stem Cells Research Institute, San Raffaele Hospital, Milano; 3) Department of Neurology, Miulli Hospital, Acquaviva delle Fonti-Bari; 4) Institute of Neurology, School of Medicine Catanzaro.

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 spastic weakness and complicated form if additional symptoms such as seizures, dementia, amyotrophy, extrapyramidal disturbance or peripheral neuropathy also occur. Most pure forms are inherited with an autosomal dominant trait, although families with an autosomal recessive or X-linked mode of inheritance have also been described. At least seven loci associated with the pure form have been demonstrated: SPG4 (2p22), SPG13 (2q24-q34), SPG8 (8q23-24), SPG3(14q11-q21), SPG10(12q13), SPG6 (15q11.1) and SPG12 (19q13). In this study, we describe the clinical findings of a multi-generation Italian kindred with SPG13. Eleven affected individuals and twenty-four unaffected subjects participated in the genetic study. Using twelve microsatellite markers linkage to chromosome 14 was detected. Significant LOD scores were obtained with D14S255, D14S978 and D14S747 with the maximum LOD score of 4.58 with D14S255. Haplotypes analysis locates the SPG13 gene between markers D14S266 and D14S281. A gene (LOC 63380), belonging to the ATPase family, is located in this region. The fourteen coding exons of this gene from a patient and a healthy control were sequenced, but no point mutations were observed in these individuals relative to the published wild-type sequence. This finding excludes this gene as causative for SPG3.



**A Novel Autosomal Recessive Pure Hereditary Spastic Paraplegia in a Saudi Family Showing linkage to Chromosome 13q.** *C.A. Hodgkinson, S.A. Bohlega, E.J. Cupler, S.N. Abu-Amero, C.E. Boumah, M. Kambouris, B.F. Meyer, V.A. Bharucha.* Biological & Medical Research, KFSH&RC, RIYADH, SAUDI ARABIA.

Hereditary spastic paraplegia (HSP) is a complex disorder arising from mutations at a larger number of different loci. Transmission of the trait can be either autosomal dominant or recessive, and can also be X-linked. HSP is usually divided by the mode of transmission (dominant or recessive), and also as "pure" and "complex". In pure HSP no other traits are associated with the development of paralysis, whereas in the case of complex HSP other traits such as mental retardation are associated with the disorder. In a consanguineous marriage two unaffected parents produced eight children. Five of the children have shown difficulty in walking since early childhood, and are severely spastic. None of these individuals displays any apparent mental retardation or any visual defects. Spastic changes appear to be limited to the lower extremities. No other affected relatives have been reported. A whole genome scan using the CHLC marker set has linked the locus for the paraplegic trait to chromosome 13q by autozygosity. No HSP locus has been previously linked to chromosome 13, and therefore this family exhibits a novel form of autosomal recessive pure HSP. The interval underlying the HSP trait has been further refined using 17 additional markers to determine the limits of the homozygous region. Based upon these data, and on mapping of marker sequences to the newly available human genome sequence, we conclude that the gene causing this novel HSP lies within a 2.8 Mega-base region.

**Mutation screening of two candidate genes: Human peptide transporter and Human glypican 5 in families affected with bipolar disorder.** *M. Maheshwari<sup>1</sup>, E.S. Gershon<sup>2</sup>, S.L. Christian<sup>2</sup>, R.A. Gibbs<sup>1</sup>*. 1) Dept Molec/Human Gen, Baylor Col Medicine, Houston, TX; 2) Dept of Psychiatry, University of Chicago, Chicago.

Schizophrenia is a complex disorder. Gene mapping studies have identified chromosome 13q32 as one of the regions with evidence of linkage to schizophrenia and bipolar disorder. Human peptide transporter (HPEPT1) and human Glypican 5(GPC5) are two of the candidate genes within approximately 10 cM region of chromosome 13q32 region. In order to find susceptibility genes, we performed mutation screening of these two genes in 7 families (n=20) affected with bipolar disorder. The genomic structures of HPEPT1 and GPC5 were determined by direct comparison of their full-length cDNA with the sequence of human chromosome 13 genomic clones. Intronic oligonucleotide primer pairs flanking the coding fragments were designed for both the genes using program primer 3. Screening of the coding region of the genes for the sequence variants was performed by PCR amplification of each coding fragment. Twenty samples of seven families with bipolar disorder and a control sample were analyzed for twenty coding fragments of HPEPT1 and seven coding fragments of GPC5 gene. PCR reactions were carried out under the standard PCR conditions. Sequencing of each sample was done using the bodipy chemistry on an ABI 3700 DNA sequencer and analysis was done using sequencer program. No mutation or polymorphisms were identified in the GPC5 gene. Two variants were discovered in HPEPT1 gene, one in fragment 16 and the other one in fragment 20. Sequence analysis revealed a T to C substitution in third position of codon encoding alanine at 1403 position (Frag. 16) of mRNA and A to G substitution at position 2242 (Frag. 20) of mRNA. Of these, 6 individuals were homozygote CC, 15 heterozygote TC (Frag. 16) and 3 were heterozygote AG (Frag. 20). In the next step we plan to do genotype association studies of observed polymorphisms in larger samples of bipolar disorder and control population.

**Linkage of bipolar disorder susceptibility loci to chromosomes 22q and 13q in two independent family sets.** *J.R. Kelsoe<sup>1</sup>, S.H. Shaw<sup>1,2</sup>, Z. Mroczkowski-Parker<sup>1,3</sup>, T. Shectman<sup>1,3</sup>, M. Alexander<sup>1,3</sup>, R.A. Remick<sup>4</sup>, A.D. Sadovnick<sup>5</sup>, S. McElroy<sup>6</sup>, P. Keck<sup>6</sup>.* 1) Department of Psychiatry, UCSD, La Jolla, CA; 2) Department of Medicine, UCSD, La Jolla, CA; 3) San Diego VA Healthcare System, La Jolla, CA; 4) Department of Psychiatry, St. Paul's Hospital, Vancouver, Canada; 5) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 6) Department of Psychiatry, University of Cincinnati, Cincinnati OH.

Bipolar disorder or manic depressive illness is a severe psychiatric disorder with 1% prevalence and strong evidence for genetic factors. We have previously reported a genome scan of bipolar disorder in a set of 20 families from the general North American population. The regions with the strongest evidence of linkage in this study were 22q and 13q. The genome-wide maximum lod score in this sample was 3.8 at D22S278 on 22q13. We now report our linkage analysis of the same markers on 22q and 13q in a new set of 32 families similarly ascertained from the North American population. This second family set includes 194 subjects, 69 of whom have bipolar I or bipolar II disorders and 59 of whom have recurrent major depression. In this second family set on 22q, a maximum lod score of 2.2 was obtained at D22S684 approximately 2 MB from D22S278. Lod scores between 1.5 were also obtained near D22S419 which was a possible secondary peak in the first study. Linkage to 13q32 was also supported in this second family sample with a lod score of 2.29 at D13S154. Analyses of the combined sample of 52 families yielded multiple markers with lod scores between 2 - 2.5 on 22q in each the two regions detected in the previous sample. On 13q, the multipoint maximum lod score peaked approximately 7 cM proximal to D13S154 (lod = 3.40) with a second peak occurring between D13S225 and D13S796 (lod = 2.58). Together, these data in a second family set are consistent with our first study, as well as, other published reports and support the presence of at least one bipolar disorder susceptibility locus on both 22q and 13q. As each of these regions have been implicated in several studies of schizophrenia, they also raise the possibility of common susceptibility loci for these two disorders.

**PARK6 IS A MAJOR LOCUS IN EARLY-ONSET PARKINSONISM.** *E.M. Valente<sup>1,2</sup>, F. Brancati<sup>1,4</sup>, A. Ferraris<sup>1,4</sup>, A.R. Bentivoglio<sup>3</sup>, V. Bonifati<sup>4</sup>, J. Vaughan<sup>2</sup>, A. Pizzuti<sup>1,4</sup>, G. De Michele<sup>5</sup>, V. Caputo<sup>1</sup>, A. Brice<sup>6</sup>, T. Gasser<sup>7</sup>, M. Breteler<sup>8</sup>, B.A. Oostra<sup>8</sup>, B. Dallapiccola<sup>1,4</sup>, N.W. Wood<sup>2</sup> and European Consortium on Genetic Susceptibility in Parkinson Disease.* 1) C.S.S. Mendel Institute, Rome, Italy; 2) Institute of Neurology, London, UK; 3) Istituto di Neurologia, Università Cattolica, Rome, Italy; 4) Università La Sapienza, Rome, Italy; 5) Dipart. di Scienze Neurologiche, Università Federico II, Naples, Italy; 6) INSERM U289, Hôpital de la Salpêtrière, Paris, France; 7) Neurologische Klinik, Ludwig Maximilians Universität, Munich, Germany; 8) Erasmus University, Rotterdam, the Netherlands.

Parkinson's disease (PD) is a common neurodegenerative disorder characterised by tremor, rigidity and bradykinesia. Autosomal recessive juvenile parkinsonism (ARJP) is a clinically and genetically distinct entity, in which common PD features are associated with early onset, good response to levodopa and early levodopa-induced dyskinesias. So far, only 1 ARJP gene (Parkin) has been cloned, and several mutations have been detected in AR families and sporadic cases. However, in at least 50% of ARJP patients, no mutation could be found in the Parkin gene. We have recently mapped a novel recessive locus (PARK6) to chromosome 1p in a large Sicilian family with ARJP. We studied 25 European ARJP families not linked to Parkin. All available family members were genotyped for markers spanning the PARK6 locus. Pairwise and multipoint LOD scores were calculated and haplotypes were constructed. We detected linkage in 8 families from 4 different countries. Reconstruction of shared haplotypes between the affected sibs from each family and determination of the smallest region of homozygosity in a consanguineous family reduced the candidate interval to 9 cM. These findings confirm the linkage of a subset of Parkin-negative ARJP families to the PARK6 locus. No common haplotypes were detected among the linked families, suggesting different independent mutational events. PARK6 appears to be a major locus for ARJP in European patients.

**MAPPING OF AUTOSOMAL RECESSIVE CHRONIC DISTAL SPINAL MUSCULAR ATROPHY TO CHROMOSOME 11q13 IN A LARGE KINDRED.** *L. Viollet<sup>1</sup>, P. Burlet<sup>1</sup>, E. Vial<sup>1</sup>, A. Barois<sup>2</sup>, G. Rebeiz<sup>3</sup>, A.*

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Distal spinal muscular atrophy (distal SMA) represents a heterogeneous group of neuromuscular disorders caused by progressive anterior horn cell degeneration causing progressive motor weakness and muscular atrophy predominantly in the distal part of the limbs. Distal SMAs are also known as spinal-type Charcot Marie Tooth disease (spinal-CMT), distal Hereditary Motor Neuropathy type II or neuronal motor neuropathy form of Peroneal Muscular Atrophy. Various forms have been reported, with autosomal dominant or recessive mode of inheritance. We report here on chronic autosomal recessive distal SMA in a large inbred family, with various ages at onset. Since this condition shared neurological features with spinal muscular atrophy with respiratory distress and diaphragmatic paralysis (SMARD) mapped to chromosome 11q13-q21, we tested the disease for linkage. We mapped the disease locus to chromosome 11q13 in the genetic interval (D11S4076 and D11S1321) which encompasses the SMARD gene ( $Z_{max}$ : 4.59 at recombination  $q = 0$  at locus D11S4136). Thus, despite differences in the age at onset, clinical course and severity, this study supports the view that both chronic distal SMA and SMARD could be allelic disorders. The occurrence of diaphragmatic paralysis in some patients further reinforces this view. The study of candidate genes of the region will hopefully lead to the identification of the disease causing mutation and will determine whether SMARD and chronic distal SMA are indeed accounted for by the same gene. However, given the substantial size of our interval (~18.6-Mb according to the Human Genome Project Working Draft at UCSC database) and the high gene density of the 11q13 region, additional pedigrees are needed to refine this genetic interval.

**Additional, physically-ordered markers increase evidence of linkage between bipolar disorder and chromosome 18q22.** *T.G. Schulze<sup>1</sup>, Y.-S. Chen<sup>1</sup>, J.A. Badner<sup>1</sup>, M.G. McInnis<sup>2</sup>, J.R. DePaulo, Jr.<sup>2</sup>, F.J. McMahon<sup>1</sup>.* 1) Psychiatry, The University of Chicago, Chicago, IL; 2) Psychiatry, Johns Hopkins University, Baltimore, MD.

Prior studies in the Johns Hopkins University / Dana Foundation Bipolar Disorder Pedigrees indicated linkage of bipolar affective disorder (BPAD) to chromosome 18q21-23. Most of the linkage evidence derived from families with affected phenotypes in only the paternal lineage and from marker alleles transmitted on the paternal chromosome. We recently confirmed this finding in an analysis of a dense set of 32 microsatellite markers in the linkage region. We furthermore observed that paternal allele-sharing across 18q21-23 was greater in families with at least 2 siblings who had a particular subtype of BPAD known as bipolar II disorder. Multipoint affected sib-pair analysis of the 81 affected sib-pairs in these families produced a peak paternal lodscore of 4.67, with an approximate 1-lod support interval of 12 male cM. We have now added 6 additional markers to the 6 markers in and near the previous 1-lod support interval. Since recombinational ordering of such a dense set of markers is prone to errors, we instead determined marker order physically, according to the available human genome sequence. The complete set of 12 markers spans a region of 5.5 Mb with a mean inter-marker distance of 1.2 male cM, twice as dense as the previous marker set. Multipoint affected sib-pair linkage analysis revealed an increase in the peak paternal lodscore from 4.67 to 5.42 (empirical  $p < 0.007$ ). Linkage resolution also increased: The approximate 1-lod support interval narrowed from 12 to 9 male cM on chromosome 18q22. Our results support our previous findings and define a relatively small region suitable for further studies aimed at identifying the genetic variation that accounts for these linkage findings. This study also illustrates how sequence information can be used to minimize errors in marker order and thus increase the information value of linkage signals in studies of complex phenotypes.

**X-Linked Bilateral Perisylvian Polymicrogyria maps to Xq.** *L. Villard<sup>1</sup>, K. Nguyen<sup>1</sup>, C. Cardoso<sup>5</sup>, C. Lese<sup>5</sup>, A. Weiss<sup>5</sup>, M. Sifry-Platt<sup>3</sup>, A.W. Grix<sup>3</sup>, J.B. Graham<sup>4</sup>, R.M. Winter<sup>2</sup>, R.J. Leventer<sup>5,6</sup>, W.B. Dobyns<sup>5,7</sup>.* 1) INSERM U491, Faculté de Médecine La Timone, Marseille 13385, France; 2) Department of Clinical and Molecular Genetics, Institute for Child Health, London, UK; 3) Department of Medical Genetics, Kaiser-Permanente Point West Medical Offices, Sacramento, CA; 4) Medical Genetics and Birth Defects, Cedars-Sinai Medical Center, Los Angeles, CA; 5) Dpt of Human Genetics,; 6) Dpt of Neurology,; 7) Dpt of Pediatrics, The University of Chicago, 928 E 58th St, Chicago, IL 60657.

Polymicrogyria (PMG) is a human brain malformation characterized by excessive gyration and an abnormal cortical cytoarchitecture. It is presumed to be a defect of cortical development affecting late neuroblast migration or neuronal organization. Although the brain distribution is variable, the majority of cases are bilateral and involve the cortex surrounding the Sylvian fissures. The most frequent manifestations include epilepsy, speech and feeding difficulties, mental retardation and spasticity. In most cases, the cortical malformation is the only birth defect. Whilst intrauterine cytomegalovirus infection and vascular compromise are likely causes of PMG, evidence has accumulated supporting a genetic etiology. We have focused our analysis on the X-linked form of perisylvian PMG based on literature reports of X-linked inheritance, skewing of sex-ratio toward males in our series of patients, a preponderance of males among multiplex families and two multiplex families with affected males related through carrier females. We have included in the analysis five families with bilateral perisylvian PMG (BPP), two with definite and three with probable X-linked inheritance. We have genotyped 40 X-linked microsatellite markers providing good coverage of the entire X chromosome. Lod scores were computed using 2-point and multipoint linkage analysis softwares. Our results show significant linkage ( $Z > 2$  at  $\theta=0$  in 2-point,  $Z > 3$  in multipoint analysis) of PMG with a small region of the long arm of the X chromosome. A detailed physical and transcriptional map of the critical region is available and candidate gene analysis is currently underway to identify the causative gene defect.

**Investigation of association of SNPs with schizophrenia in a linked region on chromosome 10p.** *S.G. Schwab<sup>1</sup>, P. Sklar<sup>2</sup>, J. Hallmayer<sup>3</sup>, M. Albus<sup>4</sup>, M. Rietschel<sup>1</sup>, B. Lerer<sup>5</sup>, D.B. Wildenauer<sup>1</sup>*. 1) Dept Psychiatry, Univ Bonn, Bonn, NRW, Germany; 2) Whitehead Institute/MIT Center for Genome Research, Cambridge, MA, USA; 3) Stanford University, Palo Alto, Ca, USA; 4) Mental State Hospital, Haar, Germany; 5) Hadassah Medical School, Jerusalem, Israel.

Evidence for a potential susceptibility locus for schizophrenia on chromosome 10p has been obtained by a genome-wide survey of 71 sib-pair families using microsatellite markers. Availability of parents in these families facilitated investigation of all used microsatellite markers for association/linkage disequilibrium in presence of linkage by the transmission disequilibrium test (TDT). Moderate P values were obtained for D10S211. MALDI TOF mass spectroscopy was used to evaluate SNPs available from the SNP Consortium TSC data base in a region of 2-3 Mb for association with the trait. Several SNPs close to a potential candidate gene, the gene for a phosphatidylinositol-4-phosphate-5-kinase (PIP5K2A), produced nominally significant P values. Four novel polymorphisms within the genomic sequence of PIP5K2A have been discovered exhibiting P values  $<.02$ . One of the SNPs (G/A,  $P = .0022$ ) was found to be located in exon 7 causing a substitution of asparagine with serine. In addition, two SNPs located approx. 500kb downstream of the PIPK2A gene yielded P values of .00056, and .017. The positive regions are being further evaluated using all available SNPs and high throughput SNP genotyping using MALDI TOF mass spectroscopy in order to fine-map the area.



**Genetic analysis of the mutilated foot (*mf*) rat: an animal model for human hereditary sensory neuropathy.** *M.M.*

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The mutilated foot (*mf*) rat, derived from the Sprague-Dawley (SD) strain, is a spontaneous mutant with an autosomal recessive neuropathy. Clinical manifestations include ataxia, anaesthesia with increased pain threshold, and progressive ulceration leading to the destruction of the feet. The pathological features are a marked reduction in the number of myelinated and unmyelinated nerve fibers in the lumbar dorsal roots, and decreased neuron numbers in the cervical and lumbar dorsal root ganglia. The number of motor axons in the *mf* rat is also decreased; however, muscle power and muscle bulk are not affected. The clinical and pathological manifestations are similar to human hereditary sensory neuropathy types I and II (HSN I and II). The aim of this study is to find the causative gene for the *mf* rat in order to help our understanding of the human hereditary sensory neuropathies.

We have initially utilized a positional cloning approach allied with a backcross strategy, to establish genetic linkage in *mf* rats. Lewis rat strains were backcrossed with *mf* rats. A total of 254 second generation rats were collected, of which 119 had the mutilated foot phenotype. A genome-wide search, using simple repeat microsatellite markers, demonstrated linkage to the distal end of rat chromosome 14. Fine mapping located the *mf* locus to a 1.5 cM region between markers *D14Got73* and *D14Got76*. Mapping studies are currently underway to further refine this interval.

**Screening of candidate genes related to myelination for mutations associated with schizophrenia.** *H.J. WILLIAMS<sup>1</sup>, N.M. WILLIAMS<sup>1</sup>, G. SPURLOCK<sup>1</sup>, N. NORTON<sup>1</sup>, K.L. DAVIS<sup>2</sup>, J.D. BUXBAUM<sup>2</sup>, V. HAROUTUNIAN<sup>2</sup>, R. SAUNDERS<sup>1</sup>, A.G. CARDNO<sup>1</sup>, G. McCARTHY<sup>1</sup>, M.C. ODOANOVAN<sup>1</sup>, M.J. OWEN<sup>1</sup>.* 1) Neuropsychiatric genetics, UWCM, Cardiff; 2) Department of Psychiatry, Mount Sinai School of Medicine, New York.

Hakak and colleagues (PNAS, 98, 8, 2001) recently used DNA microarray analysis to assay the expression levels of over 6000 genes in the postmortem dorsolateral prefrontal cortex of 12 schizophrenics and matched controls. A total of 89 genes that are involved in a range of biological processes, including synaptic plasticity and neuronal development, were reported to show an altered expression profile in schizophrenics. However, their most notable finding was the differential expression of a number of genes related to myelination (MAL, CNP, MAG, transferin gelsolin and ErbB3), suggesting a disruption in oligodendrite function in schizophrenia. We have now screened each of these 6 genes for sequence variants using DHPLC and sequencing. All identified SNPs have been genotyped by primer extension and their allele frequencies estimated in an association sample of 184 DSMIV schizophrenic patients and 184 matched controls by a method of DNA pooling based on the ABI SnaPshot assay. SNPs with a significant difference in allele frequencies were then typed in a second sample of similar size by the same pooling method. Only SNPs that yielded significant results in both samples were subsequently genotyped individually in the same sample to confirm the allelic association. Of the SNPs identified the biggest difference in allele frequency between affecteds and controls yielded a  $\chi^2$  of 5.27 ( $p=0.02$ ), however the results of the association analysis of each SNP will be presented.

**Identification and Characterization of Neuropsychiatric Disease Candidate Genes in a Region of Chromosome 1 Linked to Schizophrenia.** *A. Parker<sup>1</sup>, A. Foti<sup>1</sup>, K. Burke<sup>1</sup>, C. Reeves<sup>1</sup>, G. Barnes<sup>1</sup>, K. Glatt<sup>1</sup>, R. Martin<sup>1</sup>, M. Harmon<sup>1</sup>, K. Rinard<sup>1</sup>, S. Lewitzky<sup>1</sup>, A. Aelony<sup>1</sup>, J. Byrnes<sup>1</sup>, D. Yang<sup>1</sup>, P. DiStefano<sup>1</sup>, T. Paunio<sup>2</sup>, J. Lundquist<sup>2</sup>, L. Peltonen<sup>2,3</sup>, C. Richard III<sup>4</sup>, J. Meyer<sup>1</sup>.* 1) Dept. Human Genetics, Millennium Pharmaceuticals Inc., Cambridge, MA; 2) Dept. Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland; 3) Dept. Human Genetics, UCLA, Los Angeles, CA; 4) Genetics Institute, Wyeth-Ayerst Research, Cambridge, MA.

Availability of draft human genome sequence and large gene-expression datasets has led to reanalysis of complex trait positional cloning strategies. An accepted approach to narrowing a linkage result has been to type densely spaced markers across the linked region, followed by LD and/or haplotype sharing analyses. This strategy uses anonymous markers, treating all genes in a linked region as potential candidates; while objective, it is also resource intensive, as linkage often implicates >30 MB genomic intervals. An alternative is first to identify and minimally characterize all genes in a linked interval, followed by intensive investigation of only genes consistent with the disease hypothesis at hand.

Genome-wide linkage analysis of 445 Finnish pedigrees ascertained on a sib pair concordant for schizophrenia or schizophrenia spectrum condition revealed linkage to 1q41-q43 (results reported elsewhere). Genomic sequence, cDNA, gene expression, gene prediction, and literature databases were used to annotate the gene content of a 33 cM (35.4 MB) interval, revealing 242 putative genes. Based on these data, we ranked the genes with respect to their possible functional relevance to schizophrenia. Two genes, DISC1 and a novel CAM kinase, were selected as leading candidates for analysis in our pedigree sample. We performed detailed TaqMan expression studies of both genes using microdissected human brain tissues; one showed a specific pattern of expression consistent with the hypothesized neuroanatomy of schizophrenia. SNP genotyping experiments revealed polymorphisms in both genes with nominally significant evidence for association with schizophrenia.

**Associations between 65 polymorphisms in 36 candidate genes and cardiovascular disease-related quantitative phenotypes.** *D.L. Newman<sup>1</sup>, M. Abney<sup>1,2</sup>, M.S. McPeck<sup>1,2</sup>, H. Dytch<sup>1</sup>, M.A. Grow<sup>3</sup>, A.S. Silbergleit<sup>3</sup>, C. Carlson<sup>3</sup>, A.M. Scanu<sup>4</sup>, S. Cheng<sup>3</sup>, C. Ober<sup>1</sup>.* 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Dept Statistics, Univ Chicago, Chicago, IL; 3) Roche Molecular Systems, Inc, Alameda, CA; 4) Dept Medicine, Univ Chicago, Chicago, IL.

To identify genetic risk factors for common diseases, we studied >700 Hutterites who are derived from 64 founders and members of a 1,623-person pedigree. Exposures to environmental risk factors, such as a high fat/salt diet, are uniform in this communal society. We measured systolic (SBP) and diastolic (DBP) blood pressure, and fasting LDL, HDL, Lp(a) and triglycerides (TRI) in Hutterites <sup>3</sup>14 years old and genotyped their DNA for 65 variants in 36 genes by allele-specific hybridization to strip blots (Roche Molecular Systems, Inc). The effect of each allele on the phenotype was assessed by a novel association test developed for studies in large inbred pedigrees; the relatedness among subjects is accounted for by variance components (Abney et al., 2000, *Am J Hum Genet* 66:629). Significance, assessed by a permutation-like test, accounted for the number of tests per allele (locus-specific *P*) and the number of variants examined overall (global *P*). 6 alleles had global  $P < 0.05$  and locus-specific  $P < 0.001$ : *APOE*-E2 was associated with LDL, *CETP* -630C/-628A with HDL, *APOC3* -482C and 3175C each with TRI, *LPA* 93T with Lp(a), and *ACE* allele D with SBP. No markers reached this level of significance for DBP. Thus, independent loci influence these related phenotypes in the Hutterites and these alleles are the same as those present in outbred populations. Further, 3 chromosomal regions housing these genes, 6q (*LPA*), 11q (*APOC*), 19q (*APOE*), were identified in genome-wide screens (GWS) for the corresponding phenotypes with markers that were 2-9 cM from the candidate locus, indicating that these loci may have relatively large effects on the phenotypes. Associations on GWS were not detected for the remaining 3 traits, suggesting that even in a young founder population dense maps may be required to detect linkage disequilibrium with all susceptibility loci for common diseases. Supported by NIH HL56399 and Hoffmann-LaRoche, Inc.

**A genome wide scan for early onset primary hypertension in Scandinavian families.** *O. Melander<sup>1</sup>, K. Bengtsson<sup>1,2</sup>, M. Orho-Melander<sup>1</sup>, F. Fyhrquist<sup>3</sup>, U. Lindblad<sup>2</sup>, L. Råstam<sup>2</sup>, T. Kanninen<sup>1</sup>, P. Almgren<sup>1</sup>, L. Groop<sup>1</sup>, U.L. Hulthén<sup>1</sup>.* 1) Dept of Endocrinology, Malmö University Hospital, Malmö, Sweden; 2) Dept of Community Medicine, Malmö University Hospital, Malmö, Sweden; 3) Dept of Medicine, Helsinki University Hospital, Helsinki, Finland.

Primary hypertension is a multifactorial disorder and the importance of genetic factors increase with decreasing age at onset of the disease. The aim of the present study was to identify such genetic factors by searching for hypertension susceptibility loci. To address this we performed a genome wide scan in families with clustering of early onset primary hypertension (EOHT). **METHODS:** EOHT was defined as presence of established and pharmacologically treated primary hypertension no later than at age 50 years. Families with at least two members with EOHT were collected from six different health care centres in southern Sweden and Finland. Ninety-one families with 243 affected members were ascertained (mean 2.7; range 2-6 per family). The mean age at onset of EOHT was  $40.0 \pm 7.7$  years, age at time of study  $57.9 \pm 10.1$  years and body mass index  $27.4 \pm 4.4$  kg/m<sup>2</sup>. Another 129 individuals were genotyped to obtain phase information and to estimate allele frequencies. PCR with fluorescence labelled primers was used to genotype 363 microsatellite markers (average density approximately 10 cM). Multipoint non-parametric affected pedigree member linkage analysis was performed using GENEHUNTER v.2.0. **RESULTS:** The analysis revealed eight peaks with non-parametric lod scores (NPL) >2. Best evidence for linkage was found on chromosome 2 at 120.1 cM (NPL=2.27), chromosome 3 at 117.4 cM (NPL=2.30) and chromosome 12 at 118.5 cM (NPL=2.22). Additional NPL scores >2 were seen on chromosome 1 at 102 cM (NPL=2.17), on chromosome 14 at 80.2 cM (NPL=2.05) and at 96.9 cM (NPL=2.04), on chromosome 19 at 93 cM (NPL=2.10) and on chromosome 22 at 27.7 cM (NPL=2.12). **CONCLUSIONS:** A genome wide scan in families with early onset primary hypertension revealed putative susceptibility loci on chromosomes 1, 2, 3, 12, 14, 19 and 22.

**A genome scan of aortic root diameter (ARD) in hypertensive African Americans and Whites in the HyperGEN study.** *A.I. Lynch<sup>1</sup>, D.K. Arnett<sup>1</sup>, L.D. Atwood<sup>2</sup>, R.B. Devereux<sup>3</sup>, D.W. Kitzman<sup>4</sup>, P.N. Hopkins<sup>5</sup>, A. Oberman<sup>6</sup>, D.C. Rao<sup>7</sup>.* 1) University of Minnesota, Minneapolis, MN; 2) Boston University, Boston, MA; 3) Cornell University Medical College, New York, NY; 4) Wake Forest University, Winston-Salem, NC; 5) University of Utah, Salt Lake City, UT; 6) University of Alabama, Birmingham, AL; 7) Washington University School of Medicine, St. Louis, MO.

Variation in the structure of the aortic root is thought to have a genetic basis. For example, Marfan's syndrome, which commonly includes aortic root dilation, involves the fibrillin-1 gene on chromosome 15. It has previously been shown that the inter-individual variation in aortic root diameter (ARD) is highly heritable, particularly in African Americans. In this study, we performed a genome-wide linkage scan for ARD measured by 2-dimensional echocardiography in the Hypertension Genetic Epidemiology (HyperGEN) study, one of the four networks in the NHLBI Family Blood Pressure Program (FBPP). We used data collected on 1129 African American siblings from 504 hypertensive sibships whose mean age was 51 years, and 883 White siblings from 374 hypertensive sibships whose mean age was 60 years. Standardized residual values of ARD were calculated using a linear regression model adjusting for age, age<sup>2</sup>, and field center in the minimally adjusted model, separately in sex and race groups. Height, weight, diastolic, and systolic blood pressure were also included in the fully adjusted model. Multipoint linkage analysis was performed using the GENEHUNTER2 variance components method with 367 anonymous markers spaced an average of 9.2 cM apart. Suggestive evidence for linkage was found on chromosome 5 at 85.25 cM (marker D5S1501) in African Americans, with a maximum LOD score of 2.07 in the minimally adjusted model, which decreased to 1.22 in the fully adjusted model. Suggestive evidence for linkage was also found on chromosome 1 at 156.76 cM (between markers D1S534 and D1S1653) in Whites, with a maximum LOD score of 2.40 in the minimally adjusted model, which decreased to 2.17 in the fully adjusted model. Our findings suggest that genes present on chromosomes 1 and 5 may influence the variation in ARD.

**Genome Wide Scan for Thoracic Aortic Aneurysms / Dissections, a Clinically and Genetically Heterogeneous**

**Disorder.** *S.N. Hasham*<sup>1</sup>, *D. Guo*<sup>1</sup>, *C.J Vaughan*<sup>2</sup>, *S. Shete*<sup>3</sup>, *A. Muilenberg*<sup>4</sup>, *C.T. Basson*<sup>2</sup>, *D.M Milewicz*<sup>1</sup>. 1) Dept. of Internal Medicine, University of Texas Medical School, Houston, TX; 2) Div. of Cardiology, Dept. of Medicine, Weil Medical College of Cornell University, The New York Presbyterian Hospital; 3) Dept of Epidemiology, M.D.Anderson Cancer Centre, Houston, TX; 4) The University of Iowa,Iowa.

Thoracic aortic aneurysms/dissection (TAAs) is the most frequent fatal condition in the spectrum of chest pain syndromes. Aortic aneurysms are completely asymptomatic until a life threatening event like rupture occurs. Cystic medial degeneration, an intrinsic feature of various entities such as hypertension, aging and connective tissue disorders, has been associated with the risk of aneurysms. 20% of TAAs are familial and show an autosomal dominant mode of inheritance with variable penetrance. Our lab identified a major locus at 5q13-14 in half of the known families while Dr Basson has mapped another locus at 11q23-24. Further genetic heterogeneity was seen by the identification of 11 families not linked any known loci. Analysis of clinical phenotype indicates a more diffuse arteriopathy in family associated with 11q compared to the families linked to the 5q. TAAs in the families linked to 5q is often associated with bicuspid aortic valves. A whole genome scan was carried out in four families with similar clinical phenotype. To evaluate the significance of the genome scan, we simulated the complete analysis under the assumption of no linkage and complete linkage. Of the putative loci identified, two loci at 4q and 12q showed a LOD score of suggestive linkage with two or more markers. A 12.5cM and 18cM haplotype is seen to be segregating in these families at 4q and 12q respectively. Fine mapping was carried out at these two loci using more families and markers spaced at 1-3cM. This confirmed the putative loci at 4q, under heterogeneity. Three major families are linked neither to the new suggestive loci nor to one of the two known loci indicating further heterogeneity. . Present study identified putative third locus for TAAs and also addressed the possibility of associating the clinical phenotype with the genetic heterogeneity.

**Genome scan for cardiovascular risk factors in three populations.** *B.T. Heijmans<sup>1</sup>, M. Beekman<sup>2,3</sup>, N. Lakenberg<sup>1</sup>, H.E.D. Suchiman<sup>1</sup>, G.P. Vogler<sup>4</sup>, U. DeFaire<sup>5</sup>, J.B. Whitfield<sup>6</sup>, P. de Knijff<sup>2</sup>, C. Kluff<sup>3</sup>, G.J.B. van Ommen<sup>2</sup>, R.R. Frants<sup>2</sup>, N.L. Pedersen<sup>5</sup>, N.G. Martin<sup>7</sup>, D.I. Boomsma<sup>8</sup>, P.E. Slagboom<sup>1</sup>.* 1) Medical Statistics, Leiden University Medical Centre, The Netherlands; 2) Human Genetics, Leiden University Medical Centre, The Netherlands; 3) TNO Prevention and Health, Leiden, The Netherlands; 4) Center for Developmental and Health Genetics, Pennsylvania State University, USA; 5) Karolinska Institute, Stockholm, Sweden; 6) Royal Prince Alfred Hospital, Sydney, Australia; 7) Queensland Institute for Medical Research, Brisbane, Australia; 8) Psychology, Free University of Amsterdam, The Netherlands.

The genetic basis of cardiovascular disease (CVD) is highly complex. Less complex intermediate phenotypes can be studied instead of clinical endpoints. For CVD, such phenotypes include plasma levels of lipids and apolipoproteins, which are highly heritable. The aim of our study is to map and identify genes with a major effect on these intermediate phenotypes in the general population. We are performing a genome-wide scan in population-based samples of Dutch, Swedish and Australian dizygotic twin pairs. Genehunter was used to obtain IBD status for sibling pairs. Linkage analysis was carried out in Mx using a maximum likelihood variance components approach, which included data from untyped monozygotic twins and adjusted phenotypic data for age and sex. Initial analysis of the Dutch sample indicated suggestive linkage with LDL cholesterol for chromosome 19. When marker density was increased to 6 cM, the maximum LOD-score (MLS) for LDL cholesterol was 3.8 at 65 cM in the Dutch sample (194 DZ pairs). In the Swedish population (44 DZ pairs), the MLS was 1.4 at the same location. No linkage was observed in the Australian sample (242 DZ pairs). Fine-mapping strategies were explored and indicated a 25 cM region in the Dutch sample harboring a candidate gene, which is currently being investigated. Our results provide support for the presence of a locus on chromosome 19 contributing to the variation of LDL cholesterol levels in the Dutch and Swedish population.



**A genome-wide linkage scan for essential hypertension.** *K.T. Kristjansson<sup>1</sup>, A. Kristinsson<sup>2</sup>, A. Manolescu<sup>1</sup>, Th. Hardarson<sup>2</sup>, H. Knudsen<sup>1</sup>, S. Ingason<sup>1</sup>, A. Kong<sup>1</sup>, J. Gulcher<sup>1</sup>, K. Stefansson<sup>1</sup>.* 1) Clinical Collaborations, deCODE Genetics Inc., Reykjavik, Iceland; 2) Department of Medicine, National University Hospital (Landspítalinn), IS-108 Reykjavik, Iceland.

Hypertension defined as arterial blood pressure above 140 mmHg systolic and above 90mmHg diastolic, affects up to 20 % of the adult population and 50% of those more than 60 years of age. It is one of the principal risk factors for stroke, myocardial infarction (MI), peripheral arterial occlusive disease (PAOD), congestive heart failure (CHF), and end-stage renal disease. We performed a genome-wide scan with 930 microsatellite markers using 120 extended Icelandic families with 490 hypertensive patients. The families were identified by cross matching a list of hypertensive patients from the Hypertension Clinic of the University Hospital (Landspítalinn) in Iceland with a genealogy database of the entire Icelandic nation. We analysed the data using the Allegro program and determined statistical significance by applying affecteds-only allele-sharing methods (which does not specify any particular inheritance model) implemented. The Allegro program, a linkage program developed at deCODE Genetics, calculates lod scores based on multipoint calculations. The linkage scan gave an allele-sharing lod score of 4.0 at one specific locus. We designate this locus EH1 and report on the further mapping of this locus.

**Evidence for linkage of low HDL-cholesterol trait on 8q23 and no evidence for linkage to the ABC1 gene in Finnish low HDL families.** A. Soro<sup>1,2</sup>, P. Pajukanta<sup>1</sup>, H.E. Lilja<sup>1,3</sup>, K. Ylitalo<sup>2</sup>, J.S.A. Viikari<sup>4</sup>, M-R. Taskinen<sup>2</sup>, L. Peltonen<sup>1,3</sup>. 1) Dept of Human Genetics, UCLA, Los Angeles, CA, USA; 2) Dept of Medicine, University of Helsinki, Finland; 3) Dept of Human Molecular Genetics, NPHI, Helsinki, Finland; 4) Dept of Medicine, University of Turku, Finland.

The strong relationship between low levels of high-density lipoprotein cholesterol (HDL-C) and coronary heart disease has stimulated genetic research of this complex trait. To elucidate the genetic background of the common form of the familial low HDL-C, we collected 25 extended Finnish families with familial low HDL-C and premature CHD and carried out a genome-wide scan. We found suggestive evidence for linkage (two-point lod score above 2.0) in four chromosomal regions: 2p, 8q23, 16q24.1-24.2 and 20q13.11. The significance of these initial findings for low HDL-C was further addressed by analyzing markers in these regions in an independent study sample of 29 Finnish families with familial combined hyperlipidemia (FCHL), a trait closely overlapping with familial low HDL-C. The pooled data analysis of low HDL and FCHL study samples, using low HDL as a tested trait, provided support for linkage in three of these regions on 8q23, 16q24.1-24.2 and 20q13.11. The highest statistical evidence for linkage was observed on chromosome 8q23 (a two-point lod score of 3.9), gaining support also in the multipoint analysis. Importantly, this locus has earlier been linked to HDL-C in Mexican Americans. Further, the region on chromosome 20q has previously been linked to body adiposity, hyperinsulinemia and type 2 diabetes, suggesting a partially shared genetic background of these traits and HDL-C. In addition, a locus on chromosome 2p showed evidence for linkage only in the FCHL families, this locus being 72 cM telomeric from the 2p locus initially observed only in the low HDL families. The contribution of the ABC1 gene, regulating HDL-C levels, seemed to be excluded as a major causative locus both by linkage analysis of the 25 families, and by sequence analysis of the low HDL probands. In conclusion, our genome-wide scan provided evidence for loci on 8q23, 16q24 and 20q13 affecting the HDL-C levels in Finnish low HDL families.

**A genome-wide scan for circulating levels of fibrinogen in the Framingham Heart Study.** *Q. Yang*<sup>1</sup>, *G.H. Tofler*<sup>2</sup>, *L.A. Cupples*<sup>1</sup>, *M.G. Larson*<sup>3,4</sup>, *D. Levy*<sup>3,4</sup>, *R.B. D'Agostino*<sup>5</sup>, *C.J. O'Donnell*<sup>3,6</sup>. 1) Epidemiology & Biostatistics, Boston Univ Sch Public Hlth, Boston, MA; 2) Royal North Shore Hospital, Sydney, Australia; 3) NHLBI/Framingham Heart Study, Framingham, MA; 4) Preventive Medicine and Epidemiology, Boston Univ Sch Medicine, Boston, MA; 5) Mathematics & Statistics, Boston Univ, Boston, MA; 6) Cardiology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA.

The circulating level of fibrinogen predicts future coronary heart disease and stroke. Levels of fibrinogen are correlated among family members, suggesting a heritable component to its regulation. Variants of fibrinogen gene subunits (all subunits reside on 4q28) are associated with fibrinogen levels. However, it is unknown what role, if any, is played by genetic variants on other chromosomes in the regulation of fibrinogen in the general population. We sought to confirm that fibrinogen is heritable, and we then examined for evidence of linkage to chromosomal regions in a large population-based cohort. Fibrinogen levels were measured in family members as part of routine examinations for the original and Offspring cohorts of the Framingham Heart Study. We conducted a 10 cM genome-wide scan in 330 extended families (1702 genotyped; 1595 phenotyped [402 parents, 1193 children, 852 Parent-offspring pairs, 823 Sibling pairs]). Variance-component methods were used to estimate heritability and to perform linkage analysis using sex-specific normalized residuals, adjusted for age and for age plus other important covariates. Estimated heritability was 0.30 for the age-adjusted fibrinogen and 0.23 for multivariate-adjusted fibrinogen. The highest multipoint LOD score for fibrinogen was found on chromosome 5 (LOD score 1.32 at 183 cM from the p-terminus). The next highest LOD score was found on chromosome 2 (1.16 at 58 cM). While there is no linkage reaching genome-wide significance, these data suggest that there may be influential genetic regions on chromosomes other than chromosome 4 that contribute to circulating levels of fibrinogen. Further investigation of these regions is warranted to identify fibrinogen-regulating genes.

**Importance of a cluster analysis in the promoter region of TNF $\alpha$  for the genetic risk constellation in patients with coronary artery disease.** *U. Schagdarsurengin<sup>1</sup>, P. Greiser<sup>1</sup>, S. Schulz<sup>1</sup>, D. Rehfeld<sup>2</sup>, T. Süß<sup>2</sup>, A. Kabisch<sup>3</sup>, U. Müller-Werdan<sup>2</sup>, K. Werdan<sup>2</sup>, I. Hansmann<sup>1</sup>, C. Gläser<sup>1</sup>.* 1) Inst Human Genetics, Univ Halle, Halle, Germany; 2) Dep Intern Med, Univ Halle, Halle, Germany; 3) Inst Transf Med/Bloodbank, Univ Halle, Halle, Germany.

TNF $\alpha$  is a monocyte/macrophage derived cytokine with multiple coronary atherosclerosis affecting functions. We investigated the cluster of three polymorphisms (PM) of TNF $\alpha$  of the functionally important promoter region, the G-238A, G-308A and C-863A PM in 306 probands, 146 CAD patients (age at first MI 47.9y, 125 males) and 160 healthy controls (41.8y, 108 males). Considering the progress of coronary atherosclerosis by the age of onset, 39.6% with extremely high coronary risk had already undergone their first MI at juvenile age, before 45y. **Results:** The analysis of the clustered genotypes -863/-308/-238 in the 306 probands resulted in only four frequent clusters, the wildtype cc/gg/gg (0.44), and three types with heterozygous mutation, one on position 863 (ca/gg/gg: 0.21), another 308 (cc/ga/gg: 0.19) and last 238 (cc/gg/ga: 0.05). The CAD and the control subgroup showed comparable results. But considering the age of onset we found distinct differences in the CAD patients: the cluster cc/gg/ga was more frequent in the juvenile high risk group than in the older one (0.10 vs. 0.02, n.s.). A creation of clusters of only two PMs in the juvenile and senior patients resulted in significantly different distribution concerning 863/238 PMs caused by the high frequency of 238 mutation carriers (0.17 vs. 0.02,  $p < 0.02$ ) and likewise tendencies in the cluster 308/-238 (0.10 vs. 0.04, n.s.). The combination 863/-308 did not show significances or tendencies. The separate analysis of each PM demonstrated only a high correlation of the 238 PM to progress of CAD: the mutation carriers (GA+AA) were significantly more frequent in the juvenile high risk patient group (MI before 45y; 0.17 vs. 0.05,  $p < 0.02$ , RR 3.35). **Conclusion:** Cluster analyses should absolutely bear in mind the weight importance of each polymorphic marker in the risk constellation. Our results show the importance of the 238-promoter PM of TNF $\alpha$  as an evident genetic risk marker for CAD.

**Recent progress in identification of the X-linked infantile spinal muscular atrophy (XL-SMA) gene: Refined linkage analysis results in candidate gene investigations.** *H. Basterrecha*<sup>1</sup>, *D. Dressman*<sup>2,3</sup>, *M.E. Ahearn*<sup>1</sup>, *A. Meindl*<sup>4</sup>, *N.T. Bech-Hansen*<sup>5</sup>, *R.D. Clark*<sup>6</sup>, *B. Wirth*<sup>7</sup>, *M. Barmada*<sup>3</sup>, *E. Hoffman*<sup>2</sup>, *L. Baumbach*<sup>1</sup>. 1) Univ. Miami Med Sch, Miami, FL; 2) Children's National Med Ctr, Washington, DC; 3) Univ. Pittsburgh, Pittsburgh, PA; 4) Ludwig Maximilians Univ, Munchen, Germany; 5) Univ Calgary, Alberta, Canada; 6) USC Cancer Ctr, Los Angeles, CA; 7) Univ Bonn, Bonn, Germany.

We have previously reported an X-linked form of infantile lethal motor neuron disease (MIM 30021), which closely resembles Werdnig-Hoffman disease, except for additional features of early onset or congenital contractures and/or fractures, in a single family that mapped to Xp11.3-q11.2. We have identified 12 unrelated families from North America and Central or Western Europe. Of these, eight families have been tested for linkage on the X-chromosome; all of these map to the same region as the first family. One of these is thought to represent a new mutational event. The cumulative LOD score for the families is 7.8148 at a  $q=0.0$  in the Xp11.3-q11.2 region. Further multipoint linkage analysis has narrowed the disease gene interval to a region redefined by DXS8080-DXS7132. These results strongly support the existence of a major disease locus in this region. From the approximately 100 genes in the *XL-SMA* candidate region, potential candidate disease genes have been identified: a) Genes involved in neuronal and muscle development; b) Genes involved in cell-to-cell communication; c) Genes involved in RNA processing/gene expression; d) Genes expressed in fetal muscle or central nervous system. We are developing high-throughput mutation screening methods for candidate gene studies. Our latest results will be presented. In conclusion, we have demonstrated overwhelming evidence that an X-linked form of infantile SMA can be detected in many regions of the world; that families representing new mutations in the yet-to-be identified gene exist; and that a single disease locus resides in the pericentromeric region of the X chromosome. It is our expectation that the *XL-SMA* disease gene will be identified in the next year, allowing for molecular diagnosis of suspected cases.

**Syndromic X-linked mental retardation and hypopituitarism maps to Xq23-25.** *N.D. Leslie<sup>1</sup>, M. Keddache<sup>1</sup>, S. Chernausek<sup>2</sup>, J. Abuzzahab<sup>2</sup>.* 1) Division of Human Genetics, TCHRF 1041, Children's Hosp Research Fndn, Cincinnati, OH; 2) Division of Endocrinology, Children's Hospital Research Fndn, Cincinnati, OH.

A family is described in which mental retardation accompanied by hypopituitarism segregates as an apparent X-linked recessive trait. Six affected males in a 3 generation pedigree exhibit a phenotype with severe-moderate mental retardation associated with expressive language dysfunction and short stature. Additional features include a large forehead with a frontal cowlick, downturned mouth, short philtrum, excessive sweating, constipation, and self-abuse. The proband has growth hormone deficiency and another affected male in a previous generation experienced hypoglycemia, for which he was treated with cortisone injections. Obligate female carriers are not mentally retarded, although many have depression or bipolar affective disorder. Linkage analysis for X-chromosomal markers at 10 Cm density resulted in a maximum lod score of 2.3 with marker DXS 8055 at Xq23-25. Recombination events at flanking markers DXS 1106 and DXS 1001 defined the 20 cM critical region. X-linked panhypopituitarism has been associated with reported in association with mental retardation in families carrying a duplication of Xq26-27. In addition, mental retardation associated with bipolar disorder had been reported in a female with a balanced translocation at Xq24, interrupting the glutamate receptor 3 gene (GRIA3). In both of the previously reported families, the breakpoints mapped telomeric to the distal bound of the critical region defined in this family. Data from this family further support the hypothesis that multiple genes for syndromic mental retardation are clustered in the Xq22-27 region.

**Genetic heterogeneity in schizophrenia II: conditional analyses of affected schizophrenia sibling pairs provides evidence for an interaction between markers on chromosomes 8p and 14q.** *Y.-F. Chiu<sup>1</sup>, J.A. McGrath<sup>2</sup>, M.H. Thornquist<sup>2</sup>, P.S. Wolyniec<sup>2</sup>, G. Nestadt<sup>2</sup>, K. Swartz<sup>2</sup>, V.K. Lasseter<sup>2</sup>, K.-Y. Liang<sup>3</sup>, A.E. Pulver<sup>2</sup>.* 1) Department of Biostatistics, University of North Carolina, Chapel Hill, NC., 27599, USA; 2) Department of Psychiatry and Behavioral Sciences, The Johns Hopkins School of Medicine, Baltimore, MD 21231, USA; 3) Departments of Biostatistics and Epidemiology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21205, USA.

Information from multiple genome scans and collaborative efforts suggests that schizophrenia is a heterogeneous, complex disorder with polygenic and environmental antecedents (1). In a previous paper we demonstrated that stratification of families on the basis of co-segregating phenotypes (psychotic affective disorders [PAD] and schizophrenia spectrum personality disorders [SSPD]) in first degree relatives of schizophrenic probands increased linkage evidence in the chromosome 8p21 region (D8S1771) among families with co-segregating SSPD (2). We have now applied a method of conditional analysis of sib-pairs affected with schizophrenia, examining shared alleles identical-by-descent (IBD) at multiple loci (3). The method yields enhanced evidence for linkage to the chromosome 8p21 region conditioned upon increased allele sharing at a chromosome 14 region. The method produces a more refined estimate of the putative disease locus on chromosome 8p21, narrowing the region from 18 cM (95% confidence interval) in our previous genome scan (4), to approximately 9.6 cM. We have also shown that the affected siblings sharing 2 alleles IBD at the chromosome 8p21 region and 1 allele IBD at the chromosome 14 region differ significantly in clinical symptoms from non-sharing affected siblings. Thus the analysis of allele sharing at a putative schizophrenia susceptibility loci conditioned on allele sharing at other loci provides another important method for dealing with heterogeneity.

**The Effectiveness of Computational Methods in Haplotype Prediction.** *K.F. Lewis<sup>1</sup>, C.F. Xu<sup>1</sup>, K.L. Cantone<sup>1</sup>, P. Khan<sup>1</sup>, C. Donnelly<sup>1</sup>, N. White<sup>1</sup>, N. Crokker<sup>1</sup>, P.R. Boyd<sup>1</sup>, D.V. Zaykin<sup>2</sup>, I.J. Purvis<sup>1</sup>.* 1) Molecular Genetics, GlaxoSmithKline, Stevenage, Hertfordshire, England; 2) Population Genetics, GlaxoSmithKline, Research Triangle Park, North Carolina, USA.

Haplotype analysis has been used for narrowing down the location of disease susceptibility genes and for investigating many population processes. Conventionally, Haplotype phases are established by laborious processes, such as studies using extended pedigrees or using molecular methods. A cheap alternative strategy is to use computational algorithms to predict haplotype phases from genotype data. In this study, we have empirically evaluated the effectiveness and accuracy of such computational methods in haplotype prediction. We experimentally determined the genotypes and haplotypes of 5 single nucleotide polymorphisms (SNPs) in the N-Acetyltransferase 2 gene (NAT2, 850-bp, n=81) and 5 SNPs on chromosome X (140-kb, n=77). We applied the expectation-maximisation (EM) algorithm and the subtraction algorithm to predict haplotype frequencies and individual haplotype phases. Both algorithms provided effective and accurate estimates for haplotype frequencies and individual haplotype phases for the NAT2 region, where there was near complete linkage disequilibrium (LD) between SNPs. For the chromosome X locus, both computational methods were adequate in estimating overall haplotype frequencies. However, neither method was effective or accurate in predicting individual haplotype phases for this region, where marked LD was not maintained. We found that the EM algorithm provided a better estimate of haplotype frequency than the subtraction method for both genetic regions.



**Clinical Applications: An Interactive Clinical Data Management System For Genetic Studies.** *R.V. Jones, B.L. Chesnut, C.P. Blach, C.W. Howe, C.S. Haynes, E.R. Hauser, M.A. Pericak-Vance.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

Genetic studies in complex traits require the collection and management of large clinical datasets. The ability to integrate these clinical data with laboratory results for genetic analysis is critical for a successful outcome. Thus, we developed Clinical Applications (CA), a modular Windows-based front-end system for the PEDIGENE® database. While ensuring patient confidentiality through security measures, an intuitive user interface enables a researcher to extract clinical data from the database for a desired individual, family, group, site or the entire study for viewing or editing purposes or for generating reports. Reports list demographic, sample, general medical history, or study-specific clinical exam data in a grid-format that can be subsequently sorted, filtered, edited, printed, or saved to files for use in genetic analysis. CA also assists in quality control measures by tracking the changes made to the data and by whom. Users can also readily obtain summarizing clinical information on overall diagnostic impression as well as stratification by clinical subtypes. New features, reports, studies, or clinical exams can be incorporated into the system by adding or modifying the appropriate modules. This flexibility also allows the system to be adapted to work with other databases and applications. Using this approach, genetic analyses can be customized to take advantage of the available phenotypic information. CA has been invaluable in our genetic linkage and association studies and is a prototype system for similar studies of complex traits.

**A graphical model approach to exact calculation of multilocus genetic probabilities on large pedigrees.** *S.C. Heath.* Dept Epidem & Biostat, Mem Sloan-Kettering Cancer Ctr, New York, NY.

Calculation of genetic probabilities underlies both parametric and non-parametric genetic linkage analysis, and limitations of the algorithms to calculate these probabilities restrict the types of problems that can be analyzed. The existing algorithms either restrict analysis to simple pedigrees with few loci (Elston-Stewart), or very small pedigrees with many loci (Lander-Green). Both of these algorithms can be considered as special cases of a more general graphical model approach, developed to calculate the probability of Bayesian networks.

The advantage of the graphical model approach occurs when the observed data constrain the possible genotypes of observed individuals, such that certain genotype combinations have zero probability. This occurs, for example, with codominant markers when it is assumed that there are no marker errors. In this case, the graphical model approach can take full advantage of the constraints imposed by the data, possibly allowing for more complex problems to be analyzed than is possible using either the Elston-Stewart or the Lander-Green algorithms.

In the extreme case when every pedigree member is genotyped, multilocus calculations with >10 loci can be rapidly performed on even very large and complex pedigrees (>2000 individuals, >100 loops). In general, it is almost always possible to perform an exact calculation if everyone is genotyped, no matter how many loci and how large the pedigree (although it is not difficult to construct pathological counter-examples). In the more realistic case where several people have missing genotypes, the graphical model approach can still provide advantages over the existing algorithms, but computational requirements increase rapidly as the informativeness of the data decreases.

The application of the new graphical model approach to a range of different pedigrees (both real and simulated) will be presented, and comparisons of the computational requirements made (where possible) with existing linkage analysis packages. This work was supported by NIH grant GM58757-03.

Program Nr: 1885 from the 2001 ASHG Annual Meeting

**A Framework for the Objective Measurement of Genetic Variation.** *N.J. Markward, M.Z. Peliás, B.J.B. Keats, W.P. Fisher, Jr.* Department of Genetics, L.S.U. Health Sciences Center, New Orleans, LA.

Geneticists have mistaken numbers or numerical formulations of their observations for mathematical, interchangeable units of measurement. This assumption is incorrect and reflects a bias that has pervaded Western philosophy since Galileo: "...that the book of Nature is written in the language of mathematics." In actuality, numerical variables, including counts of nucleotide and amino acid sequences, levels of gene expression, and population allele frequencies, are premathematical in that they do not provide the common exchange currency genomic research requires for generality and inferential stability across laboratories, samples, and scientific constructs. Premathematical numbers must be converted from their context-dependent form to an abstract, mathematical language of measurement. Only then can meaningful inferences be drawn about how molecular variation influences person-specific disease susceptibility and temperament, as well as how individual-level variation contributes to population dynamics and speciation. This project demonstrates how raw genetic information may be converted to a common mathematical, sample-independent measurement scale, as mandated by a simple, eloquent mathematical law. The resulting quantities function as additive units of interchangeable value that may be plugged into any quantitative genetic formula. Implications are drawn for research theory, clinical practice, and public policy formulation.

**Mendel version 4.0: a complete package for the exact genetic analysis of discrete traits in pedigree and population data sets.** *K. Lange<sup>1,2,3</sup>, R. Cantor<sup>1</sup>, S. Horvath<sup>1,4</sup>, M. Perola<sup>1</sup>, C. Sabatti<sup>1,3</sup>, J. Sinsheimer<sup>1,2,4</sup>, E. Sobel<sup>1</sup>.*  
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The Mendel software program performs likelihood-based statistical analysis to solve a variety of genetic problems. Where appropriate, the analysis will use either the Lander-Green-Kruglyak or Elston-Stewart algorithms, whichever is more efficient for each individual pedigree. This program includes options to:

- 1) order and map a set of markers along a chromosome;
- 2) localize a disease gene on a fixed marker map,  
using either parametric or non-parametric linkage methods;
- 3) test for allelic association by the TDT or the gamete-competition model;
- 4) determine haplotypes;
- 5) identify potential genotyping errors;
- 6) determine kinship coefficients conditional on marker data;
- 7) perform genetic risk calculations;
- 8) test for paternity or other pedigree relationships;
- 9) estimate allele frequencies, with or without using the pedigree structure;
- 10) estimate trait genotype penetrances;
- 11) identify deviations from Hardy-Weinberg and linkage equilibrium; and
- 12) simulate genetic data by gene dropping.

Mendel has been completely rewritten for this new version and now includes flexible input formats. Unix and Windows versions of this program, sample analysis files, and documentation are available.

**Multiplexing of primer extension products for mass spectrometry.** *M. Wjst*<sup>1</sup>, *N. Herbon*<sup>1</sup>, *T. Bettecken*<sup>2</sup>, *T. Immervoll*<sup>1</sup>. 1) Inst fuer Epidemiologie, GSF Forsch Umwelt & Gesundh, Neuherberg Munich, Germany; 2) Inst fuer Humangenetik, GSF Forsch Umwelt & Gesundh, Neuherberg Munich, Germany.

MALDI-TOF (matrix assisted laser desorption ionisation time of flight) mass spectrometry is a fast and precise method for detecting single base variation in DNA sequences (Ross, Nature Biotechnol 1998; 16:1347). In addition it offers the advantage of combining different primer extension reactions in a single tube.

Method: We designed primers for 500 consecutive SNPs on chromosome 6p21 obtained from the SNP consortium (snp.cshl.org) with Primer3 software ([www.genome.wi.mit.edu/genome\\_software/other/primer3.html](http://www.genome.wi.mit.edu/genome_software/other/primer3.html)). Biotinylated primer for the capture PCR were set to a target size of 25 bases, PCR product size to 80 to 140 bases while extension primers should be on average 16 base long. Criteria for multiplexing were (i) annealing T<sub>m</sub> of extension primer within a 50C interval (ii) same ddNTP stopmix solution (iii) least square mass differences of the extension product weights and (iv) at least 50 D differences of extension product weights. The multiplexing program is available at [cooke.gsf.de/wjst/group/develop/snp\\_menu.cfm](http://cooke.gsf.de/wjst/group/develop/snp_menu.cfm)

Results: Automatic assay design was possible for 408 SNPs with most failures due to insufficient sequence information, followed by annealing problems within repeat regions. Due to the large resolution range of the fragments between 4500 and 7500 Dalton multiplexing was straightforward: Bipllexing resulted in 214 assays (52% of original set), triplexing in 152 (37%), quadruplexing in 127 (31%), pentaplexing in 111 (27%), hexaplexing in 99 (25%). Pentaplexing of the first 300 SNPs resulted in a ratio of 28%, 250 SNPs of 27%, 200 SNPs of 34%, 150 SNPs of 33% and 100 SNPs of 37% compared to the original set.

Conclusion: Pentaplexing of at least 250 SNPs is probably the most economic way to combine primer extension products for mass spectrometry.

**A web-based software system for linkage analysis and information management.** *G.Y. Yu, J.X. Liu, D.T. Xie, R.M. Thompson, R. Zayas, R.S. Raike, C.M. Gomez.* Dept Neurology, Univ Minnesota, Minneapolis, MN.

It has become feasible to conduct high-throughput genotyping in a regular laboratory setup due to the availability of standard microsatellite marker sets, and advances in automatic devices for DNA extraction, amplification, and electrophoresis. The challenges remain to be the information management and efficient data analysis, especially for a small laboratory. We developed a web-based software system to address the challenges. This system provides information management services and an intuitive graphic user interface (GUI) for integrated analysis programs. The core of the system is a database-backed application and a GUI that communicate over the internet. Many data analysis programs can be easily integrated. We have currently integrated LINKAGE (FASTLINK) and SLINK. Using this system, we are performing a genome-wide scan for dominantly inherited ataxia genes. It streamlines our data entry and analysis process. The system employed the three-tier client/server architecture. On the front-end, an easy-to-use GUI is presented in a web browser. An authorized user can enter data, validate Mendelian consistency, import or export formatted files, do analysis, and report results through the GUI. On the backend, there is a relational database. It stores marker information, pedigree information, clinical data, disease models, DNA sample data, and genotyping data. Users can make any combination of a pedigree set, a marker set, and a disease model to perform an analysis using any integrated program. The database also stores all the analysis information, including all input files and final results. In the middle, a Java application serves as a four-way bridge between the browser, the database, the genetic analysis programs, and the platform on which the application is running. The system is object-oriented designed and implemented in Java, and is platform- and database- independent. It thus provides flexibility, reusability, scalability and maintainability.

**Mutations in the Diastrophic Dysplasia Gene (DTDST) are not a frequent cause of Idiopathic Talipes Equinovarus (Clubfoot).** *L. Bonafe<sup>1</sup>, S.H. Blanton<sup>2</sup>, A. Scott<sup>3</sup>, A. Ward<sup>4</sup>, S. Broussard<sup>4</sup>, C.A. Wise<sup>5</sup>, A. Superta-Furga<sup>1</sup>, J.T. Hecht<sup>4</sup>.* 1) University Children's Hospital, Zurich Switzerland; 2) Univ of VA, Charlottesville; 3) Shriner's Hospital for Children, Houston, TX; 4) Univ of TX HSC, Houston, TX; 5) Scottish Rite Hospital, Dallas TX.

Idiopathic talipes equinovarus (ITEV) or isolated club foot is a common birth defect whose etiology remains elusive. Many studies suggest a genetic etiology with either a single gene or multifactorial inheritance most often invoked. A recent report suggested homozygosity for the R279W mutation in the sulfate transporter gene (DTDST; SLC26A1) as the etiology of ITEV in two sibships. We have assembled a collection of 203 sporadic parent-child trios, 72 parent-child trios with a positive family history and 48 multiplex families with ITEV. Although the affected individuals in the two reported sibships had additional anomalies, we decided to investigate a possible role for DTDST in isolated clubfoot. Using closely linked flanking markers, D5S1507 and D5S1469, in a subset of our families we tested DTDST as a candidate gene for ITEV. TDT scores gave suggestive results and therefore we sequenced the DTDST gene of ten ITEV probands who had a positive family history and also had the excessively transmitted allele. No alterations in the sequence were identified. Two R279W heterozygous individuals (1%) were found out of the 207 tested probands with ITEV; this is consistent with the frequency of the R279W mutation in a control population. Our findings suggest that the DTDST gene does not play a significant role in the etiology of ITEV.

**Toward the identification of the MKS2 gene causing the Meckel syndrome.** *Y. Sirot<sup>1</sup>, L. Benedetti-Clech<sup>1</sup>, J. Augé<sup>1</sup>, J. Roume<sup>1</sup>, V. Cormier-Daire<sup>1</sup>, M. Le Merrer<sup>1</sup>, G. Gyapay<sup>2</sup>, A. Munnich<sup>1</sup>, M. Vekemans<sup>1</sup>, T. Attié-Bitach<sup>1</sup>.* 1) Dept Genetique INSERM U393, Hopital Necker Enfants Malades, Paris, France; 2) Centre National du Séquençage, Génoscope, Evry, Paris.

Meckel syndrome (MKS) is a rare, lethal, autosomal recessive congenital malformation syndrome characterized by occipital meningoencephalocele, multicystic dysplasia of the kidney, cystic and fibrotic changes of the liver, and polydactyly. So far, two MKS loci have been mapped to chromosomes 17q21-23 and 11q13 in finnish (MKS1) and african-middle eastern families respectively (MKS2). In an attempt to identify the MKS2 gene, we constructed an integrated map across the 0 cM critical region between markers D11S4079 and Ac18610A. This map has allowed us to pursue our gene candidate analysis by RT-PCR sequencing of two patients and to exclude a third gene (PTD015). In addition, we established the structure of a novel gene by construction of a EST contig and its comparison with human genomic sequence. This gene which is conserved in the mouse, *Drosophila* and *C.Elegans* is being tested by RT-PCR sequencing. Finally, the expression patterns of PTD015 and this novel gene are being studied using In Situ Hybridization on human embryos.



**Lack of Confirmation of Linkage to Previously Reported Regions for NSCLP.** *S. Patel*<sup>1</sup>, *J. Mulliken*<sup>2</sup>, *S.H. Blanton*<sup>3</sup>, *J.T. Hecht*<sup>1</sup>. 1) UTHSC, Houston, TX; 2) Boston Children's Hospital, MS; 3) University of Virginia, Charlottesville.

Nonsyndromic cleft lip and palate (NSCLP) is a common craniofacial birth defect occurring in 1/700 livebirths. The etiology of NSCLP is complex with genetic and environmental factors playing a role. Identification of the genetic factors has been a challenge. Genome scans and candidate gene approaches have been used to identify potential NSCLP genes. Prescott et al. (2000), using sib-pair analysis, reported suggestive linkage to 9 chromosomal regions. In our study, we have tested linkage and association to markers in these regions in a collection of 72 multiplex NSCLP families. In addition, we have tested markers tightly linked to the metalloproteinase (MMPI) gene family on chromosome 11 as these genes, especially MMPI13, play an important role in cranial neural crest cell migration and cell-cell adhesion. The data was analyzed using traditional parametric linkage analysis, non-parametric linkage analysis and the TDT, all as implemented by GENEHUNTER. No evidence was found for either linkage alone or linkage and association. These results suggest that these regions do not play a major role in the development of familial NSCLP.

**Genetics of familial keloids.** *A.G. Marneros<sup>1,2</sup>, J.E.C. Norris<sup>3</sup>, B.R. Olsen<sup>1,2</sup>, E. Reichenberger<sup>1,2</sup>*. 1) Department of Cell Biology, Harvard Medical School, Boston, MA; 2) Harvard-Forsyth Department of Oral Biology, Harvard Dental School and Forsyth-Institute, Boston, MA; 3) Department of Plastic Surgery, St. Luke's/Roosevelt Hospital Center, New York City, NY.

Keloids are proliferative fibrous growths that result from an excessive tissue response to skin trauma. Most keloids occur sporadically, but some keloid cases are familial. The genetics of keloid formation have, however, only rarely been documented, and the mode of inheritance is not known. We studied the clinical and genetic characteristics of 14 pedigrees with familial keloids. The ethnicity of these families is mostly African-American (10), but also Caucasian (1), Japanese (2), and African-Caribbean (1). The pedigrees account for 341 family members, of which 98 display keloids. Of the affected family members, 38 are male, and 60 are female. The age of onset varies from early childhood to late adulthood. There is variable expression of keloids within the same families, some affected members having only minor ear lobe keloids, whereas others have very severe keloids affecting large areas of the body. In the described pedigrees, 7 individuals are obligate unaffected carriers, revealing nonpenetrance in about 6.7% of keloid gene carriers. Syndromes associated with keloids, namely Rubinstein-Taybi and Goeminne syndrome, were not found in these families. Additionally, linkage to the gene loci of these syndromes was excluded. A complete genome wide screen using 403 microsatellite markers was performed in the ten largest pedigrees. Preliminary linkage data suggest locus heterogeneity, since distinct loci with significant and suggestive linkage were identified in single families. The pattern of inheritance observed in these families is consistent with an autosomal dominant mode with incomplete clinical penetrance, and variable expressivity. This is the most comprehensive collection of keloid families described to date, and it allows for the first time the elucidation of the clinical genetics of the familial form of this wound healing disorder, and the mapping of keloid gene loci.

**Fine mapping of a diabetes susceptibility gene on chromosome 12 in Caucasian samples.** *M.G. Ehm, M.C. Karnoub, G. Chandra, S.L. Chissoe, S. Haneline, E.H. Lai, Q. Nguyen, D. Yarnall, D.V. Zaykin, M.J. Wagner, D.K. Burns.* GlaxoSmithKline, Res Triangle Pk, NC.

We undertook a genome-wide screen (389 microsatellite markers) in samples of 835 Caucasian individuals collected as part of the American Diabetes Association's GENNID study. Multipoint nonparametric linkage analyses were performed with diabetes, and diabetes or impaired glucose homeostasis (IH). We have previously reported linkage to diabetes (LOD=1.91) and diabetes or IH (LOD=2.81) near D12S853 (map position 82 cM). While these results were not replicated in samples collected in phase 2 of the GENNID study, evidence for linkage of type 2 diabetes to this region on chromosome 12 has been reported by two other investigators on independent Caucasian family samples. We identified, mapped and genotyped 551 markers at an average density of 20 Kb, in a sample of 153 unrelated cases taken from the above Caucasian families, and 133 unrelated Caucasian population controls. In this work, we will outline the statistical methods used to fine map the putative susceptibility gene in this region.

Single marker tests of association of genotypes for single markers include chi-squared tests and Hardy-Weinberg equilibrium tests (Nielsen, Ehm, Weir 1999). To assess association of haplotypes with disease, we employed a sliding window approach in conjunction with the Zaykin et al (in preparation) haplotype trend test. To investigate whether our marker map is sufficient to extract all of the information in the region, we have investigated the marker allele frequencies and summarized the extent of linkage disequilibrium. Additionally, we are investigating the information content of the haplotypes to summarize how efficiently the marker map is extracting information in the region. We have identified 6 regions for follow-up based on the results of these tests and are typing these markers in an independent Caucasian case and control collection. We are investigating whether any of these SNPs is associated with the evidence for linkage (Horikawa, Oda, Cox et al 2000).

**Linkage studies of *FASL* polymorphisms in type 1 (insulin dependent) diabetes in Danish families.** *R.L. Nolsoe, O.P. Kristiansen, Z.M. Larsen, J. Johannesen, F. Pociot, T. Mandrup-Poulsen.* Steno Diabetes Center, Gentofte, Denmark.

**Background/Aims.** Type 1 (insulin dependent) diabetes mellitus is the result of a T cell dependent selective destruction of the pancreatic beta cells. There is evidence that the apoptosis inducing T cell effector, Fas ligand (FasL) may play a role in the pathogenesis of type 1 diabetes, probably on the level of the T cell. FasL mediated apoptosis is important in maintaining peripheral self-tolerance and in down-regulating an immune response. **Methods.** We therefore evaluated the human FasL gene on chromosome 1q23 as a candidate susceptibility gene for type 1 diabetes and scanned the entire *FASL* (promoter, exons 1-4 and 3'UTR) for polymorphisms using single strand conformational polymorphism analysis and direct sequencing. **Results.** We identified two novel polymorphisms, a g-C843T and a g-A475T, in a negative regulatory region of the promoter. A Danish type 1 diabetes family collection of 1143 individuals comprising 257 families (420 affected and 252 unaffected offspring) was typed for the g-C843T polymorphism and for a *FASL* microsatellite. Haplotypes were established and data were analysed using the extended transmission disequilibrium test, ETDT. We found no significant deviation from random transmission patterns for the haplotypes, ( $P_{etdt}=0.20$ ). **Conclusion.** We found no overall evidence for linkage of the *FASL* polymorphism to type 1 diabetes and conclude that *FASL* does not contribute to the genetic susceptibility to type 1 diabetes.

**Localizing genes involved type 1 and type 2 diabetes in a genetically isolated population.** *C.M. Van Duijn<sup>1</sup>, N. Vaessen<sup>1</sup>, J. Pullen<sup>2</sup>, J.J. Houwing-Duistermaat<sup>1</sup>, P. Snijders<sup>1</sup>, L.A. Sandkuijl<sup>1</sup>, M. Edwards<sup>2</sup>, P. Heutink<sup>1</sup>, B.A. Oostra<sup>1</sup>.* 1) Genetic Epidemiology Unit, Dept Epidemiology & Biostatistics and Clinical Genetics, Erasmus Univ Medical Sch, Rotterdam, Netherlands; 2) Oxagen, UK.

Type 1 (DM1) and type 2 (DM2) diabetes have a substantial genetic component. The HLA-DR/DQ region and the insulin gene are involved in DM1 while in DM2 calpain-10 has been implicated. For both diseases, other genes must be involved. The aim of the present study was to identify new loci involved in diabetes in a genetically isolated population. In the middle of the 18th century, 150 settlers founded the population in Soutwestern part of the Netherlands, which now comprises 20,000 inhabitants. Of the 46 DM1 patients ascertained, 43 could be linked to a common ancestor within 15 generations. The number of HLA-DR3/DR4 carriers in patients was significantly lower compared to that of DM1 patients from the general Dutch population ( $p=0.0001$ ) suggesting other loci may be more important in our isolate. The genome wide screen using 420 markers showed a peak at D6S1014 located in the HLA-region with a p-value of 0.009. Based on this finding we used a p-value of 0.016 (=LOD score 1.0) as a cutoff in the 2 genome screens. Using this criterion, there was evidence for a DM-1 locus reported earlier at chromosome 8q (D81128; $p=0.003$ ) and a new locus on chromosome 17q (D17S2059; $p=0.012$ ). Both loci showed a conserved haplotype in patients ( $p$ -trend=0.0005 for both markers). The 128 DM2 patients who were ascertained could be aggregated into 79 nuclear families linked to each other within 13 generations. There was no evidence for a role of calpain-10 in our isolate. Of the 770 used in the genome screen, 9 yielded a lodscore over 1.0. To replicate the initial findings of the genome screen, an independent study of serum glucose, a risk factor for DM2, in first degree relatives was conducted. For 2 loci, one yielding a LOD score 2.3 in the initial DM2 screen, the relation between the conserved DM2 haplotype and serum glucose was replicated in relatives. Although the genes involved in DM1 and DM2 loci remain to be identified, our initial results suggest that the population is suitable for localization of new diabetes genes.

**Role of resistin sequence variation in type 2 diabetes and insulin resistance.** *H. Wang, W. Chu, C. Hemphill, S.C. Elbein.* Division of Endocrinology, Univ Arkansas for Medical Sciences, Little Rock, AR.

Insulin resistance in fat and muscle characterizes type 2 diabetes (T2DM). Resistin, a novel cysteine-rich signaling molecule, is secreted by adipose cells and is increased in genetic and diet-induced models of obesity and insulin resistance. Insulin sensitivity improves when resistin levels fall. Thus, resistin may explain the connection between insulin resistance and obesity. We hypothesized that sequence variation in or near the resistin gene might account for observed differences in insulin sensitivity and diabetes risk among nondiabetic members of families with multiple T2DM siblings. We used available sequence data to predict the genomic structure and designed primers to screen 800 bp of 5' flanking region and 2.5 kb of intron and coding regions, including the 3' flanking region, using single strand conformation polymorphism analysis (SSCP) under 2 conditions. We tested 44 unrelated diabetic individuals and 20 nondiabetic individuals at extremes of insulin sensitivity from 63 families of Northern European ancestry ascertained for two individuals with T2DM. Variants were confirmed by fluorescent bidirectional sequencing. We identified one 5' flanking SNP, 2 3' flanking SNPs and a trinucleotide repeat, and 5 intronic SNPs, but no coding SNP. We typed the 5' flanking SNP and one intronic SNP in 129 unrelated cases (T2DM) and 118 unrelated nondiabetic controls, and in 122 nondiabetic family members (96 normal, 26 impaired glucose tolerance) who had undergone minimal model determination of insulin sensitivity. Neither variant was associated with T2DM. Neither variant affected insulin sensitivity with or without body mass index (BMI) as a covariate. Likewise, no effect was found on BMI as a trait. The two variants were in significant linkage disequilibrium ( $p < 0.001$ ). Work is in progress to type additional variants that are not in complete linkage disequilibrium. To our knowledge, this is the first analysis of resistin variation in humans. Our data suggest that resistin variants are unlikely to alter diabetes risk or insulin sensitivity in Caucasians, but variation might be important in other ethnic groups or in a more obese population.

**Linkage analysis of chromosome 13 in families selected for specific language impairment.** *C.W. Bartlett<sup>1</sup>, J. Flax<sup>1</sup>, P. Tallal<sup>1</sup>, L.M. Brzustowicz<sup>1,2,3</sup>*. 1) Dept Neurosci/Ctr M&BN, Rutgers Univ, Newark, NJ; 2) Dept Genetics, Rutgers Univ, New Brunswick, NJ; 3) New Jersey Medical School, Newark, NJ.

Specific language impairment (SLI) is a developmental disorder characterized by a failure to learn language normally, in absence of specific environmental and neurological explanations. In the process of a genome scan, five nuclear and extended families (N=58) with at least two affected individuals were genotyped at 10 cM intervals spanning chromosome 13. Three phenotypic classifications with both dominant and recessive models were tested with parametric linkage analysis: 1) Clinical Impairment; 2) Reading Impairment; and 3) Language Impairment. The analysis was completed in three steps. First, pairwise linkage analysis was conducted on all markers with one marker giving a lod score  $> 1.5$  at D13S800 in 13q21. 3-point analysis of the same region gave a lod score  $> 2$ . Next, four additional markers were genotyped in the region at approximately 5 cM intervals. Finally, 4-point analysis of the region gave a lod score of 2.68 ( $\alpha = 1$ ) close to marker D13S800 under a recessive Reading Impairment model. This region corresponds to the same location in previous work in autism where subsetting multiplex families based on language criteria increased the evidence for linkage in this region. These results suggest the most likely location of a putative SLI locus is located near D13S800 and may represent the same locus seen in autism research.

**Further evidence for linkage of autosomal dominant medullary cystic kidney disease (ADMCKD) on chromosome 1q21.** *S. Ala-Mello*<sup>1</sup>, *M. Auranen*<sup>2</sup>, *J.A. Turunen*<sup>2</sup>, *I. Järvelä*<sup>2,3</sup>. 1) Dept Medical Genetics, Univ Helsinki, Helsinki, Finland; 2) Dept of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 3) Helsinki University Central Hospital, HUCH Laboratory Diagnostics, Laboratory of Molecular Medicine, Helsinki, Finland.

**Background.** Autosomal dominant medullary cystic kidney disease (ADMCKD) is characterized by the development of cysts at the corticomedullary border of the kidneys, it resembles nephronophthisis (NPH) with an autosomal recessive mode of inheritance. Genetic linkage has been shown either on chromosome 1q21 (ADMCKD1) or 16p12 (ADMCKD2), and there exist families that are not linked to the aforementioned loci. No disease-causing gene underlying this disorder has been reported.

**Methods.** We searched the Finnish Transplantation Register and hospital records to identify all the ADMCKD families in the Finnish population. Detailed clinical information of the patients was collected. Linkage analysis was used to study whether the Finnish families originating from a homogenous population showed genetic linkage to the ADMCKD1 or ADMCKD2 loci. Also, the coding region of a strong candidate gene, natriuretic peptide receptor A (NPRA) located on the chromosome 1q21 critical region was sequenced.

**Results.** Five of the six families showed linkage to the previously identified region of chromosome 1q21. Family 6 with hyperuricemia as a prominent clinical feature was linked to neither of the ADMCKD loci. Wide inter- and intrafamilial variability in the clinical picture of the patients was detected. The NPRA gene mutation was excluded as a causative gene by sequencing.

**Conclusion.** This study locates the gene for ADMCKD1 close to a marker D1S1595 in a region < 5cM, and further confirms the existence of at least three loci for the medullary cystic kidney disease. Heterogeneity of the symptoms complicates the clinical diagnosis and classification of the patients. Further studies are needed to identify the disease-causing gene.



**Linkage of quantitative endophenotypes in autistic sibpairs.** *M. Alarcón*<sup>1</sup>, *R.M. Cantor*<sup>2</sup>, *AGRE Consortium*<sup>3</sup>, *D.H. Geschwind*<sup>1</sup>. 1) UCLA Department of Neurology, Los Angeles, CA; 2) UCLA Department of Human Genetics, Los Angeles, CA; 3) Cure Autism Now, Los Angeles, CA.

Autism is characterized by repetitive behaviors and deficits in language and social skills. Linkage analyses of the autism diagnosis have been performed but results have not been consistent. Its apparent complex genetic etiology suggests that associated quantitative endophenotypes may be important in identifying linkage. To identify quantitative trait loci (QTL) that influence autism-related traits, we performed a nonparametric multipoint genome scan analysis in 152 families from the Autism Genetic Resource Exchange (AGRE; Geschwind et al., in press). The families were ascertained for the presence of at least two children with autism spectrum disorders based on the Autism Diagnostic Interview (ADI-R; C. Lord et al., 1994). Three quantitative traits from the ADI-R were analyzed: age at first word, age at first phrase and a composite measure of repetitive or stereotyped behavior, which we call ocd. Parents and offspring were genotyped for 335 markers and multipoint sibpair analyses were conducted using Genehunter (L. Kruglyak et al., 1996). The strongest evidence supporting linkage for the ADI-R item 'age at first word' was observed on chromosome 7q ( $Z = 2.98$ ,  $p=0.001$ ). This region is about 25 cM away from the SPCH1 locus (S. E. Fisher et al., 1998) and less than 3 cM away from a previously reported autism susceptibility region (International Molecular Genetic Study of Autism Consortium, 1998). The result for language delay was supported by a subsequent multipoint analysis of additional markers in the chromosome 7q region ( $Z=2.85$ ,  $p=0.002$ ). Moreover, the peak fine mapping result for ocd ( $Z=2.48$ ,  $p=0.007$ ) was in a region overlapping the language QTL. These findings suggest that the putative susceptibility gene on chromosome 7 may be a QTL for the language and repetitive behavior deficits associated with autism.

**Mixture models for linkage and association analysis with covariates.** *B. Devlin<sup>1</sup>, S-A. Bacanu<sup>1</sup>, B.L. Jones<sup>2</sup>, K.M. Roeder<sup>3</sup>*. 1) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Genetics, Univ Pittsburgh, Pittsburgh, PA; 3) Dept Statistics, Carnegie Mellon University, Pittsburgh, PA.

One of the challenges to determining the genetic etiology of complex diseases is genetic heterogeneity. Covariates, such as attributes or environmental exposures of the subjects, can be used to focus tests on more homogeneous subsets of the data to detect linkage (Schaid et al., 2001, *Am J Hum Genet* 68:1189-1196; Devlin et al., *Genet Epidemiol*, in press) or association. For example, age of onset was a key covariate for the discovery of BCRA1; fasting insulin and body-mass index could be key covariates for determining the genetic risk factors for Type 2 diabetes. For ASP linkage analysis, we formulated the problem as a mixture model in which a disease mutation is segregating in only a fraction of the ASP, with the remaining fraction being unlinked. Covariate information is used to predict membership within two groups, corresponding to the linked and unlinked ASP. Then tests for linkage are conducted using all families, each family being weighted by the probability of membership in the cluster of interest. We are currently extending the mixture model approach to association analysis, especially family-based analysis. Here the covariate information is again used to cluster families into two groups, which correspond to families deriving at least a portion of their liability from alleles at a candidate locus (i.e., linked and associated families) versus those families having the disease for other reasons. Because the structure of the linkage and association models is similar, we expect the theoretical framework to extend naturally. We evaluate the performance of these methods by simulation.

**Genome-wide multipoint linkage analyses of five extended multiplex pedigrees from the Oceanic Nation of Palau.**

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The Oceanic Nation of Palau has been geographically and culturally isolated over most of its 2000 year history. In our early studies of schizophrenia in Palauans, we selected five large, multigenerational pedigrees for linkage analysis and genotyped markers every 10 cM (CHLC/Weber screening set 6). The number of affected/unaffected individuals genotyped per family ranged from 11/21 to 5/5. We fit a simple dominant and recessive model to these data using multipoint linkage analysis implemented by SIMWALK2. As might be expected, the most informative pedigrees produced the best linkage results. After genotyping additional markers in the region, one such pedigree produced a LOD = 3.4 (5q distal) under the dominant model. Seven of 9 schizophrenics in the pedigree, mostly third and fourth degree relatives, share a 15 cM, 7 marker haplotype. For a different pedigree, another promising signal occurred on distal 3q, LOD = 2.6 for the recessive model. For two other pedigrees, the best LODs were modest, slightly better than 2.0 on 5q and 9p, while the fifth pedigree produced no noteworthy linkage signal. Currently, all available schizophrenics have been ascertained (n=154 of 156) along with key relatives. (n=495). Using this completely ascertained population of schizophrenic families, a genome wide scan has been performed at the Center for Inherited Diseases (CIDR) genotyping facility using microsatellite markers spaced every 10 megabases. Genome wide multipoint results will be presented based on these new data.

**Evidence of genetic heterogeneity in Seckel syndrome.** *L. Faivre<sup>1</sup>, M. Le Merrer<sup>1</sup>, S. Lyonnet<sup>1</sup>, A. Verloes<sup>2</sup>, N. Dagonneau<sup>1</sup>, B.C. Xavier<sup>1</sup>, A. Munnich<sup>1</sup>, V. Cormier-Daire<sup>1</sup>.* 1) Dept Genetics, Hopital Necker Enfants Malade, Paris, France; 2) Medical Genetics Centre, CHU Sart Tilman, Liege, Belgique.

Seckel syndrome is a rare autosomal recessive condition belonging to the group of osteodysplastic primordial dwarfism. This syndrome is characterized by the association of severe pre and postnatal growth retardation, microcephaly with mental retardation and a typical bird-headed facial appearance. This syndrome has been suggested to be clinically heterogeneous. Recently, a locus for Seckel syndrome has been mapped to chromosome 3q22.1-q24 by homozygosity mapping in two consanguineous families originating from the same village in Pakistan. Here, we exclude linkage to chromosome 3q22.1-q24 in five consanguineous families originating from Europe (1), North Africa (3) and Africa (1) using 6 microsatellite markers of the region of interest distant from 5 cM (D3S1309 to D3S1282 from the centromere to the telomere). These results support the view that Seckel syndrome is a genetically heterogeneous condition. Studies of additional families with this condition will help to define whether or not this locus is a major locus for Seckel syndrome, and if genetic heterogeneity is related to phenotypic or ethnical differences. Furthermore, we also exclude linkage to chromosome 3q22.1-q24 in two multiplex consanguineous families presenting type II osteodysplastic primordial dwarfism.

**Localization of the Human SLE Susceptibility Genes Within the HLA Using a Recombinant Ancestral Haplotype Approach.** *R.R. Graham<sup>1</sup>, W.A. Ortmann<sup>1</sup>, P.M. Gaffney<sup>1</sup>, S.A. Selby<sup>1</sup>, P.E. Stuart<sup>2</sup>, J.T. Elder<sup>2</sup>, P.K. Gregersen<sup>3</sup>, T.W. Behrens<sup>1</sup>.* 1) Medicine, University of Minnesota, Minneapolis, MN; 2) Dermatology, University of Michigan, Ann Arbor, MI; 3) Genetics, North Shore University Hospital, Manhasset, NY.

The Human Leukocyte Antigen (HLA) region on Chromosome 6p21.3 is a primary genetic susceptibility locus in many autoimmune diseases, including Systemic Lupus Erythematosus (SLE). Previous studies in SLE have been unable to precisely localize this effect due to the extensive linkage disequilibrium in the HLA, a paucity of informative markers, small sample sizes, and the limitations of case/control approaches. The recent sequencing of the HLA region has allowed us to develop a dense map of highly informative microsatellite markers spanning this 3.6 Mb region. Here, we report the genotyping of 44 markers in the Minnesota SLE family collection, which is comprised of 334, primarily Caucasian, families. Analyses of the collected marker data demonstrates that the HLA region confers susceptibility to SLE as measured by tests for both linkage (LOD>3.5) and association (Pedigree Disequilibrium Test [PDT] global p value =  $3 \times 10^{-8}$  at M6S115). We found that two extended HLA haplotypes, the 8.1 and 7.1 ancestral haplotypes bearing the HLA-DR2/DQ6 and HLA-DR3/DQ2 alleles, respectively, are strongly associated with disease and account for most of the observed effect. The extensive disequilibrium present at the HLA has the potential to make the localization of disease genes a challenge. Indeed, we found that more than half of the 8.1 and 7.1 founder haplotypes in our population span at least 2.2 Mb. A recombinant ancestral haplotype approach was employed to better localize the susceptibility genes present on the 8.1 and 7.1 haplotypes. This approach has narrowed the relevant region of the 8.1 (-DR3) haplotype to an ~1.1 Mb (MICA-M6S230) interval, while the susceptibility region of the 7.1 (-DR2) haplotype was localized to an ~400 kb region in the Class II region (M6S137-M6S232). These data have allowed us to significantly narrow the SLE disease-gene containing intervals within the HLA and should facilitate gene identification.

**A transmission disequilibrium test that allows for genotyping errors in the analysis of single nucleotide**

**polymorphism data.** *D. Gordon*<sup>1</sup>, *S.C. Heath*<sup>2</sup>, *X. Liu*<sup>1</sup>, *J. Ott*<sup>1</sup>. 1) Lab Statistical Genetics, Rockefeller Univ, New York, NY; 2) Lab Mathematical Genetics Memorial Sloan-Kettering Cancer Center, New York, NY.

Our work assesses the effects of genotyping errors on the type I error rate of a particular transmission disequilibrium test (TDTstd) that assumes data are errorless, and introduces a new transmission disequilibrium test (TDTae) that allows for random genotyping errors. We evaluate the type I error rate and power of the TDTae under a variety of simulations, and perform a power comparison between the TDTstd and the TDTae for errorless data. Both the TDTstd and the TDTae statistics are computed as 2 times a log-likelihood difference, and both are asymptotically distributed as chisquare with 1 df. Genotype data for trios are simulated under a null hypothesis, and under an alternative (power) hypothesis. For each simulation, errors are introduced randomly under a specific model via a computer algorithm with different probabilities (called allelic error rates). The TDTstd statistic is computed on all trios that show Mendelian consistency, while the TDTae statistic is computed on all trios. The results indicate that TDTstd shows a significant increase in type I error when applied to data in which inconsistent trios are removed. This type I error increases both with an increase in sample size and an increase in the allelic error rates. TDTae always maintains correct type I error rates for the simulations considered. Furthermore, TDTae shows power of at least 0.958 for several simulations. Finally, the power of TDTstd is at least that of TDTae for simulations with errorless data.

**Genomic Screen for Genes Responsible for Early Onset Open Angle Glaucoma.** *E.A. DelBono<sup>1</sup>, S. Lynch<sup>1</sup>, G. Yanagi<sup>1</sup>, M. Maselli<sup>1</sup>, Y. Bradford<sup>2</sup>, J.L. Haines<sup>2</sup>, J.L. Wiggs<sup>1</sup>.* 1) Ophthalmology, Mass Eye and Ear Infirmary, Boston, MA; 2) Program in Human Genetics, Vanderbilt School of Medicine, Nashville, TN.

One gene responsible for early onset primary open angle glaucoma has been cloned (TIGR/Myocilin). However, previous studies have shown that only 8-20% of families affected by dominant juvenile glaucoma have mutations in this gene. Using a collection of autosomal dominant juvenile glaucoma families without mutations in the TIGR/Myocilin gene, we have performed a genome-wide screen to identify the chromosomal locations of additional genes responsible for this condition. Twenty-five pedigrees consisting of a minimum of three affected individuals in two generations (198 total individuals, 105 affected) were used for this study. Juvenile glaucoma was defined as: age of diagnosis before age 35, IOP greater than 22 in both eyes, glaucomatous optic nerve damage in both eyes, and visual field loss in at least one eye. Individuals with secondary causes of glaucoma, including evidence of anterior segment dysgenesis and pigment dispersion syndrome, were excluded. For the initial genome screen, only affected pedigree members and spouses were included in the analysis. Two hundred thirty eight markers spanning the human genome at approximately 10 cM intervals were analyzed. Two-point lod scores were calculated using an autosomal dominant model. Five regions on chromosomes 3, 5, 9, 12 and 20 demonstrated initially interesting results (two point lod score >1.0). Multipoint analyses of chromosomes 5, 9 and 20 resulted in higher lod scores for markers located in these regions. None of these regions correspond to previously identified glaucoma loci. These initial results identify five chromosomal regions that may contain genes responsible for juvenile open angle glaucoma. Supported by Research to Prevent Blindness and NIH Grant EY09847.

**Selective taste blindness to caffeine and sucrose octa acetate: Novel bimodal taste distributions unrelated to PROP and PTC.** *P.A.S. Breslin, C.D. Tharp, D.R. Reed.* Monell Chemical Senses Ctr, Philadelphia, PA.

As part of larger project to identify individuals who demonstrate a total inability to taste bitterness, we also have observed a frequent inability to detect and rate bitterness from selected compounds, especially sucrose octaacetate (SOA) and caffeine. SOA and caffeine insensitivity appear commonly in the population at large (>30%). Our goal is to identify the genes responsible for SOA and/or caffeine blindness in these subjects by linkage analyses. This SOA/caffeine study has the advantage of examining a human phenotype that parallels the well-characterized mouse SOA insensitivity phenotype. Historically, it has been very rare to find bimodal bitterness frequency histograms for compounds that are not structurally related to phenylthiocarbamide (PTC) and propylthiouracil (PROP). SOA and caffeine are not unimodally distributed, but rather display either a bimodal or trimodal distribution. Previously, Whitney and colleagues looked for bimodality in the human frequency histogram for SOA detection thresholds, but did not find it. He was motivated by the fact that the mouse *Soa* gene influences threshold behavioral sensitivity and he wanted to determine if humans had a similar polymorphism. It is essential to note that Whitney and colleagues were measuring detection thresholds for SOA and not suprathreshold bitterness ratings as we are. It is common for detection thresholds and suprathreshold ratings not to correlate well. It is fortuitous that we have found a non-normal distribution for SOA, since we may now attempt to determine if a similar gene to *Soa* is involved with human ratings of SOA and perhaps also with ratings of caffeine. The pattern of family correlations for SOA is different than for overall bitterness sensitivity, with high parent-offspring and high sibling correlations, suggesting a greater contribution of additive gene effects. The difference in correlations between SOA and caffeine among parent-offspring and sibling groupings suggests that SOA and caffeine are likely following different modes of inheritance, and therefore supports our inference that the SOA and caffeine receptors are not the same gene. Supported by DC2995: PASB & DC04188: DRR.



**A Multiple Threshold Liability Model Suggests Linkage of Alcohol Dependence to Three Loci in the COGA Data Set.** *J. Corbett<sup>1</sup>, N.L. Saccone<sup>1</sup>, L. Bierut<sup>1</sup>, A. Goate<sup>1</sup>, H.J. Edenberg<sup>2,3</sup>, J. Nurnberger<sup>2</sup>, H. Begleiter<sup>4</sup>, T. Reich<sup>1</sup>, J.P. Rice<sup>1</sup>.* 1) Department of Psychiatry, Washington University, St. Louis, MO; 2) Department of Medical and Molecular Genetics, Indiana University, Indianapolis, IN; 3) Department of Biochemistry and Molecular Biology, Indiana University, Indianapolis, IN; 4) Department of Psychiatry, Suny Health Science Center, Brooklyn, NY.

Previous analyses of the Collaborative Study on the Genetics of Alcoholism (COGA) data set using Affected Sib Pair (ASP) methods have yielded suggestive linkage to alcohol dependence on regions of chromosomes 1, 2, and 7. These analyses used one or more diagnoses of alcohol dependence (DSM-III-R + Feigner Definite (COGA), DSM-IV, or ICD-10) as an affectation criterion. Suggestive linkage has also been reported on chromosome 4 for related phenotypes, including "Maximum Number of Drinks in a 24 Hour Period" ("Max Drinks"), "Pure Unaffected," and certain ERP phenotypes. We propose the use of a Multiple Threshold Liability Model to take advantage of the hierarchical nature of the alcohol dependence diagnoses in the COGA dataset. Taking the estimated liability values from this model, we performed a linkage analysis using a variance components method implemented in MX. There were three regions whose lod scores exceeded 1.5. The first such region, on chromosome 1, yielded a lod score equivalent of 2.59 at the marker D1S532. This is in the region of linkage for alcohol dependence previously reported in the COGA dataset. The second such region was found on chromosome 4, where there was a lod score equivalent of 1.73 near the ADH3 locus. While not in a region of previously reported linkage for alcohol dependence, it is at the same marker where a signal was reported for "Max Drinks," as well as very near the possible protective locus found using "Pure Unaffected." The final result was on chromosome 8 at the marker D8S1988 with a lod score equivalent of 2.16. While there have been no previous reports of linkage to alcohol phenotypes in this region, it is homologous to a region on rat chromosome 5 which has been shown to have linkage to an alcohol consumption trait.

**Towards localizing the gene encoding Friedreich Ataxia 2.** *J.K. Brock<sup>1</sup>, J.M. Dooley<sup>1</sup>, D.C. Riddell<sup>2</sup>*. 1) Neurology, IWK Health Center, Halifax, NS, Canada; 2) Pathology, IWK Health Center, Halifax, NS, Canada.

Friedreich Ataxia (FRDA) is an autosomal recessive progressive neurodegenerative disease. Classical FRDA is characterized by onset before 20 years of age, progressive ataxia, dysarthria, loss of deep tendon reflexes, with pyramidal tract dysfunction and sensory axonal neuropathy. Although variable in expression, most clinically described FRDA maps to human chromosome 9q13. Exceptions are a clinically similar phenotype which is associated with selective vitamin E deficiency (AVED) mapping to chromosome 8q13, and a second, clinically indistinguishable phenotype (FRDA2), which has not yet been mapped. We have identified 2 FRDA families in which the ataxic phenotype does not map to 9q13, and in which Vitamin E levels are normal, ruling out AVED. Based on work in yeast, three genes have been identified which interact with frataxin, (produced from the FRDA gene), and could represent reasonable candidates for the second FRDA gene. PCR-based linkage analysis using an intragenic marker and three flanking markers from the human homologue of one of these (the MIPEP gene) have been done. The results are partially informative and indicate that the ataxic phenotype in both of our unusual FRDA families segregates with the MIPEP gene region. Based on the number of family members studied, the odds of obtaining these results due to random segregation would be 1 in 473 (LOD score of 2.68). Although a LOD score of 3 is considered evidence of tight linkage, we still feel that MIPEP is a reasonable candidate and additional markers and family members are being examined to support this interpretation.

**Identification of gene locations from maximum likelihood ASP linkage analysis: What lessons can we learn from peak shape?** *M.P. Bass<sup>1</sup>, E.R. Martin<sup>1</sup>, M. Boehnke<sup>2</sup>, E.R. Hauser<sup>1</sup>*. 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Univ of Michigan, Ann Arbor, MI.

Estimates of disease gene location resulting from ASP linkage analysis are often imprecise, with large standard deviations (Roberts et al.,1999) and variation dependent on both sample features and genetic map characteristics. In previous simulation studies we have shown that two estimates of disease gene location generated from the same model and sample can result in location estimates as much as 20 cM apart, on average (Hauser et al.,1998). Furthermore, the shape of the maximum likelihood curves can be multimodal with large dips between regions showing interesting linkage results, say a lod score above 1 in a complex disorder. Typically follow-up studies of interesting linkage results focus attention on regions with lod scores above a certain threshold level. We used simulation studies to examine how often the disease locus can occur in a dip in the likelihood curve, first for an ideal situation with all parents typed, correctly specified map parameters and no genotyping error. Then we varied the family structure, map and marker specifications and genotyping error rate to gauge the effects on lod score curve shape. In the ideal situation for a variety of critical lod score values, we observed substantial numbers of replicates with the disease locus in the dip and the situation becomes worse as we move from the ideal situation. We discuss characteristics of the peaks when the disease locus is in the dip and examine features of these lod score curves.

**Cleft lip with or without cleft palate in Turkey: assessment of candidate regions.** *M.E. Cooper<sup>1</sup>, L.L. Field<sup>2</sup>, B.S. Maher<sup>1</sup>, R. Tobias<sup>2</sup>, G. Tunçbilek<sup>3</sup>, G. Gürsu<sup>3</sup>, M.L. Marazita<sup>1</sup>.* 1) School of Dental Medicine, Univ. of Pittsburgh, Pittsburgh, PA; 2) Univ. of British Columbia, Vancouver, BC, Canada; 3) Hacettepe Univ., Ankara, Turkey.

Cleft lip with or without cleft palate (CL/P) is a common congenital anomaly, with birth prevalences ranging from 1/500 to 1/1000 depending on the population. A number of genetic loci have shown positive linkage or association results in European Caucasian populations. The purpose of the current study was to assess whether any of those loci have positive results in Turkish Caucasian CL/P families. 14 affected individuals whose parents were consanguineous (all first cousin matings) were identified as part of our on-going studies of oral-facial clefts in Ankara, Turkey. 9 markers from chromosomes 2, 4, 6, 14, 17 and 19 were assessed (TGFA, D4S175, F13A1, D6S291, TGFB3, D17S250, D17S579, D19S49, APOC2) because each have positive linkage and/or association results with CL/P reported. LOD scores were calculated between each of the 9 markers and CL/P, as well as model-free allelic association statistics (TDT). For the LOD score calculations, an autosomal recessive model was assumed for the inheritance of CL/P. All calculations were done in the total dataset, plus in a CL subset (5 families whose probands had cleft lip alone) and a CLP subset (9 families whose probands had cleft lip plus cleft palate). Of the 10 markers, a significantly positive TDT result was obtained with TGFA in the total dataset ( $p=0.03$ ), as well as the CL ( $p=0.05$ ), and CLP ( $p=0.03$ ) subsets. None of the markers showed statistically significant positive LOD scores with CL/P, although TGFA had LOD's of approximately 1.0 in the total dataset and the CLP subset. An association with TGFA in Caucasian families was the first positive association reported for CL/P, although there have since been conflicting studies reported. This is the first study investigating these candidate regions with CL/P in Turkey. Supported by NIH grant DE12472.

**Autosomal recessive Charcot Marie Tooth families linked to the 1q21 locus.** *H. AZZEDINE<sup>1</sup>, G. Durosier<sup>1</sup>, M. Tazir<sup>2</sup>, N. Birouk<sup>3</sup>, A. Bouhouch<sup>3</sup>, M. Salih<sup>4</sup>, T. Hamadouche<sup>5</sup>, D. Mahmoudi<sup>6</sup>, A. Masmoudi<sup>6</sup>, A. Vandenberg<sup>7</sup>, E. Dubourg<sup>8</sup>, D. Grid<sup>9</sup>, A. Brice<sup>1</sup>, E. Leguern<sup>1</sup>.* 1) INSERM U289, Hopital de la Salpetriere Paris France; 2) Service de Neurologie Hopital Mustapha Algiers Algeria; 3) Laboratoire de Neurogenetique Hopital des specialites Rabat Morocco; 4) Department of Pediatrics College of Medecine & KKUH King Saud University Saudi Arabia; 5) Institut Pasteur Algiers Algeria; 6) Service de Neurologie Hopital Mayot Algiers Algeria; 7) Universite Rockefeller Lyon1 Hopital de l'antiquaille Lyon, France; 8) Unite Fonctionnelle de Pathologies Neuromusculaires Hopital de la Salpetriere Paris France; 9) AFM-Genethon, Hopital de la Salpetriere Paris, France.

Charcot-Marie-Tooth (CMT) disease is a pathologically and genetically heterogeneous group of hereditary motor and sensory neuropathies characterised by slowly progressive weakness and atrophy, primarily in peroneal and distal leg muscles. Two major types have been distinguished, in which the neuropathy is either demyelinating or neuronal. Electrophysiological studies on median motor conduction velocity (MNCV) have confirmed the distinction between the demyelinating and axonal forms of the disease. Several loci and various modes of inheritance were described: Autosomal Dominant, X linked and Autosomal Recessive CMT (ARCMT). The first locus for an axonal form of ARCMT2 was identified on chromosome 1q21 in a large consanguineous family from Morocco (Bouhouche et al., 1999). In order to refine the candidate interval, we selected 34 consanguineous families representing 125 subjects, 45 of whom are affected. All these individuals were screened using 9 polymorphic markers (D1S514, S2858, S2715, S2777, S2721, S2624, S506, S2435 and D1S2721) covering 15.4 cM including the 1.7 cM candidate region. Only 3 families from North Africa shows an homozygosity region compatible with linkage of these families to this locus. Indeed, a 4.2 maximal combined lod score was reached with marker D1S2721. The patients present with a phenotype similar to those in the morrocan family. In conclusion, we report 3 new families linked to the 1q21 locus. This form of ARCMT represents 9% of the families in our series.

**Fine-Scale SNP Genotyping for Autism using Single Base Extension and Tag Arrays.** *L. Crawford, S. Smalley, S.F. Nelson.* Dept Human Genetics, UCLA, Los Angeles, CA.

Autism is a complex neurodevelopmental disorder with an incidence perhaps as high as 1 in 500 that causes severe lifelong deficits in social and language skills. There is little understanding of the biological or genetic mechanisms underlying this disorder, although there is persuasive evidence of a genetic component. Several genes (e.g.: HTT, HOXA1, and VIPR2) and chromosomal regions ( e.g.: chr 4p, 7q, 16p) have been implicated due to candidate gene studies and several genome scans. Single Nucleotide Polymorphisms (SNPs) are particularly promising tools for localizing genes for complex disorders because they are so abundant (with 1 per several hundred bases). Tens of thousands of SNPs are in coding regions and may themselves alter gene function. We are using SNP's to 1) densely fine map regions implicated in Autism (chromosomes 7 and 16) , 2 ) more densely cover genes that have been associated with Autism to support or refute these findings and 3) study a broad selection of brain expressed genes containing coding and/or functionally relevant SNPs. Our Autism sample set has been collected at the UCLA NPI and consists of 88 affected individuals (36 from simplex families). We have found that an SBE (Single Based Extension) Tag Array addresses the need for low cost per genotype, flexibility of format and high throughput. In large-scale SNP genotyping, it is also important to reduce the high costs of identifying SNPs that are sufficiently polymorphic from those listed in dbSNP . We have found that many are either not real SNPs or are not sufficiently polymorphic. We will discuss reliable selection criteria for SNPs as well as discuss details of the SBE assay. Our SBE tag array currently allows 16 fold multiplexing for the initial PCR and 40 fold multiplexing during the single-base extension step. We are able to genotype 16 individuals for 400 SNPs at a time for a total of 6,400 genotypes per slide. Since the tags on the array do not change it is easy to utilize the same array for genotyping candidate genes or fine mapping.

Program Nr: 1913 from the 2001 ASHG Annual Meeting

**Weighting schemes in conditional model-free linkage analysis.** *e. della-chiesa, m. martinez.* EMI 00-06, INSERM, Evry, France.

Weighting schemes have recently been proposed for model-free linkage analysis of complex traits. These conditional approaches allow to map two trait loci to two separate genetic markers. Evidence for linkage at a region is assessed by weighting families to their evidence for linkage at a first region [Kong and Cox 1997]. Here, we have used both simulated and real data to investigate the effects of weighting schemes on the outcomes of model-free linkage analysis. The methodological problems we have investigated are (1) test procedures (2) derivations of weights and (3) incorporation of between-family variability in linkage information. In the simulated family data, we derived type I and type II error rates of different weighting schemes by multipoint allele-sharing linkage analyses [Kong and Cox 1997] with the package program Allegro. Weighting schemes in conditional model-free linkage analysis were also evaluated for mapping the putative non-HLA DR Rheumatoid Arthritis susceptibility gene(s) in our European genome scan (ECRAF).

**A novel Locus for autosomal dominant nuclear cataract mapped to chromosome 2p12-2q13 in a Pakistani family.**

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Congenital cataract is one of the commonest disorder of the eye in infants. Cataract may occur as an isolated anomaly or as part of a multisystem syndrome. Congenital cataracts can occur sporadically but most are familial. Familial cataracts are clinically and genetically heterogeneous and are mainly inherited in an autosomal dominant fashion with a high degree of penetrance. On the basis of whole genome search, here we report the mapping of a novel locus for autosomal dominant congenital nuclear cataract (adNCat) to 2p12 in a Pakistani family. The maximum lod score ( $Z = 4.19, q = 0$ ) was obtained for the marker D2S2333. Distal and proximal crossovers were observed with markers D2S286 and D2S1790 respectively. These crossovers define the critical disease interval to approximately 9cM. A genome database analysis of the target interval is being undertaken to identify candidate genes for the disease.



**Pedigree selection may increase genetic homogeneity in complex phenotypes: an example from lupus with hemolytic anemia.** *J. Kelly<sup>1</sup>, J. Kilpatrick<sup>1</sup>, S.K. Nath<sup>1</sup>, B. Namjou-Khales<sup>1</sup>, G.R. Bruner<sup>1</sup>, R.H. Scofield<sup>1,2,3</sup>, J.B. Harley<sup>1,2,3</sup>.* 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) University of Oklahoma, Oklahoma City, OK; 3) U.S. Dept. of Veterans Affairs Medical Center, Oklahoma City, OK.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder of unknown etiology. The hematologic abnormalities (hemolytic anemia, leukopenia, lymphopenia and thrombocytopenia) in SLE are classic manifestations of the disease. We evaluated hemolytic anemia as a selection criterion in an effort to identify a more homogenous set of pedigrees multiplex for SLE and, hence, to reveal genetic effects not previously detected. From our collection of 160 pedigrees multiplex for SLE, we analyzed a subset of 35 pedigrees (16 African-American (AA), 17 European-American (EA) and 2 Hispanic) that contained at least one SLE patient who also suffered from hemolytic anemia. Both non-parametric and maximum-likelihood model-based genome-wide linkage analyses were conducted using 328 microsatellite markers (Weber #8). In accordance with the recommendations for significant ( $LOD_{Est}^{33.3}$ ,  $p \leq 0.00002$ ) and suggestive linkage ( $LOD_{Sug}^{31.9}$ ,  $p \leq 0.00017$ ), significant linkage to hemolytic anemia was identified at 11q14 in the AA pedigrees ( $LOD_{max}=5.22$ ), and at 1q24 ( $LOD=4.00$ ) in the EA pedigrees. In addition, eight regions reached or surpassed the criteria for suggestive linkage. These are linkages important in lupus because they were identified in pedigrees multiplex for this phenotype. Their relationship to hemolytic anemia is, at present, unknown. Also, pedigree selection in SLE using this additional criterion appears to identify more genetically homogeneous pedigrees. Indeed, the 11q14 linkage in 16 AA pedigrees is quantitatively much more impressive than any linkage identified to date using large collections of pedigrees with SLE alone as the phenotype. This example demonstrates that analyzing families selected by their clinical manifestations increases the power to detect linkage for this complex disease and shows, again, the extraordinary power that clinical features have to inform disease genetics.

**Linkage Analysis in Dominant Intermediate Charcot-Marie-Tooth Neuropathy (DI-CMT).** *M.L. Kennerson<sup>1,2</sup>, D. Zhu<sup>1</sup>, R.J.M. Gardner<sup>3</sup>, E. Storey<sup>4</sup>, J. Meroroy<sup>5</sup>, G.A. Nicholson<sup>1,2</sup>.* 1) Neurobiology Laboratory, Anzac Research Institute, Concord Hospital, Concord, New South Wales, Australia; 2) Molecular Medicine Laboratory, Concord Hospital, Concord, New South Wales, Australia; 3) Genetic Health Service Victoria, Royal Children's Hospital, Victoria, Australia; 4) Department of Neurosciences, Monash University, Victoria, Australia; 5) Austin and Repatriation Hospital, Victoria, Australia.

Charcot-Marie-Tooth (CMT) neuropathy is one of the most common groups of human hereditary disorders. The CMT syndrome includes many hereditary disorders of peripheral nerve affecting both motor and sensory neurones. Two major clinical types have been defined according to whether they are primarily disorders of Schwann cells with nerve conduction slowing (CMT I) or disorders of distal portions of neurones 'axonal neuropathies' (CMT II). Motor nerve conduction velocities (NCVs) in the most common form of CMT type I (CMT1A) is typically about 20 m/sec and usually less than 40 m/sec. In axonal neuropathies (CMT II) the median NCVs are usually faster. The term 'intermediate conduction velocity' has been used to describe families with NCVs in different affected individuals in both the CMT I and CMT II ranges. For this study we have used the value of 45 m/sec to define the cut off between type I and II. We have identified a family whose NCVs in different affected individuals range from 24-54 m/sec. Segregation of the disease is consistent with autosomal dominant inheritance and we have called this form of CMT, Dominant Intermediate Charcot-Marie-Tooth neuropathy (DI-CMT). To map the gene for DI-CMT thirty-nine members of the family were recruited including 7 affected females and 5 affected males. A genome wide screen using markers located at 10 cM intervals was undertaken. Two point linkage analysis has shown the most likely location for the gene causing DI-CMT to lie in the 19p12-p13.2 region ( $Z_{max}=5.58$  at  $\theta=0.0$ ). Further studies to refine the locus are continuing to facilitate positional cloning strategies and possible positional candidate genes are undergoing mutation screening.

**Novel mutation in the ryanodine receptor associated with Malignant Hyperthermia** . *S. McWilliams*<sup>1</sup>, *T. Nelson*<sup>2</sup>, *R.T. Sudo*<sup>3</sup>, *N. Sambuughin*<sup>1</sup>. 1) Laboratory of Neuromuscular Disorders, Barrow Neurological Institute, Phoenix, AZ; 2) Department of Anesthesiology, Wake Forest University, Winston-Salem, NC; 3) Departamento de Farmacologia Basica e Clinica, ICB, Universidade Federal do Rio de Janeiro, Brazil. .

Malignant Hyperthermia (MH) is an autosomal dominant disorder that predisposes susceptible individuals to a potentially life-threatening crisis when exposed to specific commonly used anesthetic agents. During anesthesia, affected individuals experience a sudden hypermetabolic reaction characterized by increased body temperature, cardiac distress, muscle rigidity, and biochemical imbalances. These effects are thought to be caused by the abnormal regulation of calcium through the calcium release channel, ryanodine receptor (RYR1), in skeletal muscle. Mutations in the RYR1 gene account for over 50% of all known MH associated mutations. Linkage analysis of the RYR1 gene region at chromosome 19q13 was performed in a large Brazilian family with MH and all affected members of this family showed identical haplotypes cosegregating with MH. Subsequent sequencing of RYR1 gene mutational hot spots identified a novel nucleotide substitution C to T at 7063 nucleotide position, causing an amino acid change from Arg to Cys at 2355 in the ryanodine receptor. This amino acid change is cosegregated with MH in the Brazilian family and involves a conserved region among different types of ryanodine receptors. Further population studies will address the incidence of this mutation and its impact on the diagnosis of MH.

**A locus for dominant Hereditary Spastic Ataxia.** *I.A. Meijer<sup>1</sup>, C.K. Hand<sup>1</sup>, K.K. Grewal<sup>2</sup>, M.G. Stefanelli<sup>2</sup>, E.J. Ives<sup>2</sup>, G.A. Rouleau<sup>1</sup>.* 1) Centre for Research in Neuroscience, McGill University, Montreal General Hospital Research Institute, Montreal, H3G-1A4, Canada; 2) Discipline of Medical Genetics and Division of Neurology, Faculty of Medicine, Health Sciences Centre, Memorial University, St. John's, Newfoundland, A1B-3V6, Canada.

Hereditary spastic ataxia is a term used to describe a heterogeneous group of neurodegenerative disorders characterized by progressive lower limb spasticity, ataxia (dysarthria, nystagmus and impaired ocular movements), and occasionally dementia. We collected two large families (family 13 and family 71) from Newfoundland with adult onset autosomal dominant hereditary spastic ataxia presenting with leg spasticity, head jerk, dysarthria, dysphagia and eye movement abnormalities. A genome wide scan was performed on family 71 and the results showed strong evidence for linkage ( $Z_{\max} 3.26$  at  $q = 0.06$ ). Analysis of the second family, family 13, confirmed linkage ( $Z_{\max} 7.2$  at  $q = 0$ ) to this locus. Extensive haplotype analysis using polymorphic markers was performed and a shared haplotype spanning 13.6 cM was established. Key recombinants in each family refine the disease locus to approximately 5.1 cM. Several interesting candidate genes are reported in the critical region and will be considered for mutation screening. This is the first report of a dominant spastic ataxia locus.

**A new Spinocerebellar Ataxia, SCA 15.** *M.A. KNIGHT<sup>1,2</sup>, M. KENNERSON<sup>3,4</sup>, G.A. NICHOLSON<sup>3,4</sup>, R.J.M. GARDNER<sup>1,5</sup>, E. STOREY<sup>5,6</sup>, P.Q. THOMAS<sup>1</sup>, S.M. FORREST<sup>1,2</sup>.* 1) MURDOCH CHILDRENS RESEARCH INSTITUTE, ROYAL CHILDREN'S HOSPITAL, MELBOURNE, VIC; 2) DEPARTMENT OF PAEDIATRICS, UNIVERSITY OF MELBOURNE, MELBOURNE, VIC; 3) DEPARTMENT OF MOLECULAR MEDICINE, CONCORD HOSPITAL, SYDNEY, N. S. W; 4) NEUROBIOLOGY LABORATORY, ANZAC RESEARCH INSTITUTE, CONCORD HOSPITAL, SYDNEY, SYDNEY, N. S. W; 5) GENETIC HEALTH SERVICES VICTORIA, MELBOURNE, VIC; 6) DEPARTMENT OF NEUROSCIENCES, MONASH UNIVERSITY, ALFRED HOSPITAL CAMPUS, MELBOURNE, VIC.

We have studied a large Australian kindred with a dominantly inherited "pure" cerebellar ataxia. A notable observation has been the very slow rate of progression in some family members. The atrophy affects predominantly the superior vermis, but the hemispheres may be involved to a lesser extent. Pedigree information does not permit a firm conclusion as to whether or not anticipation exists. Before performing a whole genome wide scan, gene testing excluded the known spinocerebellar ataxia (SCA) loci for the following SCAs 1, 2, 3, 6, 7 and 8, and molecular linkage analysis excluded SCAs 4, 5, 6, 10, 11, 12, 13, 14 and 16. Thus we hypothesised that this might represent a new SCA clinical phenotype. It was evident that the family had no male-to-male transmission. No linkage was detected using markers on the X chromosome thus necessitating the performance of a full genome scan. Simulation analysis of the pedigree indicated a maximum LOD score of 4.4 with the power to exclude at an average of 5.4-cM either side of any marker. Analysis of the results from the whole genome scan at a 10-cM level suggested two novel areas of linkage, one at 3pter-3p24.2 and the other at the q arm telomere of chromosome 17. Multipoint analysis indicated that the chromosome 17 locus was a false positive. The candidate locus is located on chromosome 3pter-3p24.2 and has been designated as SCA15. The multipoint LOD score maps the disease to a 30-cM interval with a peak LOD score of 3.5. Candidate genes identified using bioinformatics are currently being screened to identify the disease causing mutation.

**Fine Mapping of a second locus involved in susceptibility for systemic lupus erythematosus on chromosome 4p13 in Icelandic pedigrees.** *C.M. Johansson<sup>1</sup>, K. Steinsson<sup>2</sup>, H. Kristjánsdóttir<sup>2</sup>, G. Gröndal<sup>2</sup>, M.E. Alarcón-Riquelme<sup>1</sup>*. 1) Genetics and Pathology, Rudbeck laboratory, Uppsala University, Uppsala, Sweden; 2) Department of Rheumatology, Center for Rheumatology Research, Landspítalinn, University Hospital Reykjavik, Iceland.

We have previously published a genomescan (Lindqvist, et al, *J Autoimmunity*, 14:169,2000) on Icelandic multicase families with systemic lupus erythematosus (SLE). We identified a region on chromosome 4p13 linked to SLE. The Maximum lod score (MLS) using a recessive model was  $Z=3.20$  for marker D4S1627, found in 6 extended pedigrees. The present investigation revealed a MLS of  $Z=3.94$  with the original marker for 10 Icelandic pedigrees. Several other microsatellite markers located close to D4S1627 show lod scores between 1.0 and 3.6 delimiting the region to about 5cM. Haplotype analyses have made it possible to delimit the region even further. Analysis of families from other geographical locations did not reveal evidence for linkage. This suggests either of two possibilities: a) that this locus is important in SLE in Icelandic families and potentially the result of a founder effect, or b) that our linkage is a false positive result. Nevertheless, multipoint analysis increases the lod score, supporting the presence of this locus. This locus is potentially syntenic to a mouse lupus susceptibility locus described in a cross of (MRL/lpr X C57Bl6/lpr) mice called *lmb2*. Further studies will reveal whether this region indeed harbour a SLE susceptibility gene or not.

**Genome Search for QTL Controlling Pulse Pressure: A Practical Application of the Unified Haseman-Elston**

**Algorithm.** *J. Li*<sup>1</sup>, *T. Niu*<sup>1,2</sup>, *X. Xu*<sup>1</sup>, *J. Rogus*<sup>3</sup>, *J. Yang*<sup>4</sup>, *N. Schork*<sup>1,5,6,7</sup>, *Z. Fang*<sup>4</sup>, *X. Xu*<sup>1,4,8</sup>. 1) Prog Population Genetics, Harvard Sch Public Health, Boston, MA; 2) Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 3) Section on Genetics and Epidemiology, Joslin Diabetes Center, One Joslin Place, Boston, MA; 4) Institute for Biomedicine, Anhui Medical University, Hefei, China; 5) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 6) Genset Corporation, La Jolla, CA; 7) Jackson Laboratory, Bar Harbor, ME; 8) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

The Haseman-Elston (H-E) method has played an important role in sibpair linkage analysis. To systematically search for genetic loci that regulate pulse pressure (an independent predictor for cardiocascular diseases from diastolic pressure), we scanned the entire autosomal genome using 647 sibpairs ascertained for extreme blood pressure. We applied the unified H-E algorithm in linkage analysis. Using the computer program XWXW, we detected a suggestive linkage on chromosome 2 near *D2S405-D2S1788* ( $Z=3.19$ ,  $P=0.0007$ ), a region previously linked to SBP. This linkage signal persisted after controlling for the influence of diastolic blood pressure (DBP). Possible positional quantitative trait locus (QTL) candidates include *CALM2* (calmodulin 2) at 2p21.3-p21.1, *NCX1* (Na<sup>+</sup>-Ca<sup>2+</sup> Exchanger 1) at 2p23-p22, and *POMC* (pro-opiomelanocortin) at 2p23.3. Another region on chromosome 22 near *D22S420-Mfd313* also provided suggestive evidence of linkage by the unified H-E procedure ( $Z=3.38$ ;  $P=0.0004$ ). Possible QTL candidates include *ADORA2A* (adenosine A2a receptor) and *COMT* (catechol-O-methyltransferase), both were at 22q11.2. Our results revealed that putative QTLs for PP may be located on chromosomes 2 and 22, and that in practical scenarios, the unified H-E method is efficient for mapping QTLs.

**Paget disease of bone: mapping of two loci at 5q35-tel and 5q31 and genetic heterogeneity.** *J. Morissette<sup>1</sup>, N. Laurin<sup>1</sup>, J.P. Brown<sup>2</sup>, A. Lemainque<sup>3</sup>, A. Duchesne<sup>1</sup>, D. Huot<sup>4</sup>, Y. Lacourcière<sup>5</sup>, G. Drapeau<sup>6</sup>, J. Verreault<sup>7</sup>, V. Raymond<sup>1</sup>.* 1) Molec Endocrinology, CHUL Res Ctr, Quebec, QC, Canada; 2) Rheumatology-Immunology, CHUL Res Ctr; 3) Centre national de génotypage, Evry, France; 4) Thetford Mines, PQ, Canada; 5) CHUL Res Ctr; 6) Victoriaville, Qc, Canada; 7) CUSE, Sherbrooke, PQ, Canada.

Paget disease of bone is characterized by focal increases of the bone remodeling process. It is the 2nd most common metabolic bone disease after osteoporosis. Genetic factors play a major role in the disease and two loci have been mapped for the disorder: PDB1 at 6p and PDB2 at 18q21-q22. The gene(s) causing the disorder still remain to be characterized. To decipher the molecular basis of Paget disease of bone, we performed genetic linkage analysis in 24 large French-Canadian families (479 individuals) in which the disorder was segregating as an autosomal dominant trait. A genome-wide scan with 379 microsatellites was performed on the 3 most informative family nuclei. Regions with LOD score values between 1.0 and 3.0 were assessed by higher-density mapping and haplotype analysis in the extended families. Genetic heterogeneity was investigated using HOMOG. We report strong evidence for linkage at chromosome 5q35-tel in 8 of the kindreds. Significant evidence for heterogeneity was observed at D5S408 ( $c^2 = 3.86$ ;  $p < 0.05$ ). Under heterogeneity, the maximum LOD score value of 8.71 was obtained at D5S2073. The same characteristic haplotype was shown by all pagetic patients in these 8 families, suggesting a founder effect. Recombination event confined the disease region to a 6 cM interval between D5S469 and the telomere. The 16 other families were used to map a 2nd locus at 5q31. Under heterogeneity, a maximum LOD score value of 3.70 was detected at D5S500 with  $q = 0.00$  ( $c^2 = 10.13$ ;  $p < 0.0002$ ). Key recombination events in the 2 families refined the 5q31 disease region to a 11.2 cM interval between D5S642 and D5S1972. Finally, 2 other kindreds were excluded from both the 5q35-qter and 5q31 loci. These observations demonstrate the mapping of 2 novel loci for Paget's disease of bone and provide further evidence for the genetic heterogeneity of this disorder.



Program Nr: 1923 from the 2001 ASHG Annual Meeting

**Tests of Allele Sharing and Allelic Association for Affected Sib Pairs.** *L. Lazzeroni.* Health Research and Policy, Stanford University, Stanford, CA.

Affected sib pair (ASP) data contain information about allele-sharing and allelic association, either of which can point to the presence of a disease-related gene. I will introduce a generalized ASP test that can be tailored to the combination of sharing and association likely to exist in a given setting. The proposed test can substantially increase the power to detect anticipated alternative hypotheses at little or no cost for less likely alternatives. The test is valid under most models of genetic risk, disease etiology and genotype-haplotype frequencies. When the test does detect a disease-related locus, the results offer clues about the possible contributions of the observed and linked loci that can be useful in deciding how to proceed.

**Mapping of autosomal recessive microcephaly in Indian families.** A. Kumar<sup>1</sup>, S. Rao<sup>1</sup>, A.K. Hanumanthajah<sup>1</sup>, S.C. Girimaji<sup>2</sup>. 1) MRDG, Indian Institute of Science, Bangalore, India; 2) Department of Psychiatry, National Institute of Mental Health and Neurosciences, Bangalore, India.

Microcephaly is defined as a condition in which the head circumference of an affected individual is  $>3$  SD below the mean for age. Microcephaly occurs due to a variety of reasons including intrauterine infections, drugs taken during pregnancy, maternal phenylketonuria, cytogenetic abnormalities, single-gene disorders and syndromes of as-yet-undetermined etiology. True or primary microcephaly (MCPH, MIM 251200) appears to be a distinct subtype. It is defined by the absence of associated malformations and of secondary or environmental causes. It is inherited as an autosomal recessive trait. Mental retardation in true microcephaly ranges from mild to severe, but other neurological deficits are absent. True microcephaly shows genetic heterogeneity with five known loci: MCPH1 on chromosome 8p22-pter, MCPH2 on chromosome 19q13.1, MCPH3 on chromosome 9q34, MCPH4 on chromosome 15q and MCPH5 on chromosome 1q31. We have ascertained five consanguineous families with true microcephaly from the state of Karnataka, India. On examination, the head circumferences of the affected individuals were found to be  $>5-7$  SD below the population age-related mean. All the affected individuals had mild to severe mental retardation and none were able to read and write, but could speak and had basic self-care skills. To establish linkage of these five Indian families to known MCPH loci, microsatellite markers were selected from each of the five known MCPH loci and used to genotype the families. The results showed that all but one families were not linked to known MCPH loci as alleles were not homozygous at each polymorphic marker locus examined. The only exception, family IISC-2 showed homozygosity of alleles (identical by descent) at markers D8S1819, D8S277 and D8S1825 in the affected individual, suggesting linkage of this family to MCPH1 locus. We are in the process of determining the extent of homozygous region in this family using additional microsatellite markers. The work is in progress to establish linkage of other four families using a genome-wide linkage screen. (Supported by SDTC grant PC11014).

**A potentially novel form of Batten's disease in Portugal.** *L. Huo*<sup>1</sup>, *C.A. Teixeira*<sup>2,4</sup>, *M.M. Barmada*<sup>3</sup>, *M.G. Ribeiro*<sup>4,5</sup>, *M.C.S. Miranda*<sup>4,5</sup>, *D.A. Stephan*<sup>1</sup>, *R.M.N. Boustany*<sup>2</sup>. 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Departments of Pediatrics and Neurobiology, Duke University Medical Center, Durham, NC 27710; 3) Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 4) Genetic Neurobiology Department, Institute of Cellular and Molecular Biology, University of Porto, Portugal; 5) Enzymology Unit, institute of Medical genetics, Porto.

The neuronal ceroid lipofuscinoses (NCLs), commonly referred to as Batten's disease, are a group of neurodegenerative disorders. Clinically, NCLs are characterized by rapid deterioration of vision, seizures, progressive development of mental retardation, movement disorders and behavioral changes. Incidence of the NCLs is approximately 1 to 5 in 100,000 births in the general population. The NCLs comprise eight forms classified initially by clinical symptoms and age at onset. Mutations in genes causing CLN1, 2, 3, 5 and 8 have been identified. We have identified a cohort of families from Portugal with a clinical presentation similar to CLN6. In addition, founders from Portugal settled Costa Rica, where the CLN6 gene was identified. Thus, we tested the hypothesis that CLN6 causes disease in our Portuguese families by linkage analysis. The CLN6 locus has been mapped to chromosome 15q21-23 to 4 cM between markers D15S125 and D15S1000. We analyzed 7 Portuguese families using 4 polymorphic markers within the critical region on 15. There was no evidence of linkage in any of the families, and no evidence for excess allele sharing between families at this locus (max lod score is -6.16 at D15S983). We also screened for the clinically similar mutations in CLN1,2 and 3 genes, but none were identified. These negative results suggest that we may have uncovered a novel Batten variant.

**Transmission disequilibrium analysis for a schizophrenia susceptibility locus on chromosome 18p. M.K.**

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The region of chromosome 18 short arm, especially 18p11.2, is reported as one of schizophrenia susceptibility loci. Recently, we have cloned a novel myo-inositol monophosphatase, IMPA2, from 18p11.2, identified several genetic variants, and found a significant association between the gene and schizophrenia in a case control study. In this study, we aimed to systemically narrow down a susceptibility region and confirm an existence of locus conferring a risk to the disease on 18p short arm, by means of family-based association analysis and multi-stage genetic analyses. We analyzed 119 Japanese nuclear families with schizophrenia, using 27 microsatellite markers on the 18p and proximal q-arm spacing at ~5cM, and TDT/S-TDT statistics. This first stage screening revealed the marker D18S53 on 18p11.2, which gave a significant P-value of 0.0124. Then we examined the 18p11.2 region, using additional 10 markers densely spacing at 0.4 Mb in average. In this second stage screening, we detected two markers, D18S40 and D18S852, which showed significant distortion of transmission in schizophrenia offsprings ( $P = 0.0184$  and  $0.0196$ , respectively). For the third stage, we tested the three significant markers (D18S53, D18S40 and D18S852), using the independent case (containing 130 schizophrenics) and control (containing 150 individuals) panel. In this analysis, only D18S852 still remained to have a significant allelic association. These results suggest that a schizophrenia predisposing gene may be located in the 18p11.2 region in Japanese cohorts. We are now further analyzing this region using single nucleotide polymorphisms, and by performing haplotype transmission analysis to obtain a firm evidence of association.

**Linkage analysis in autosomal dominant congenital cataract families.** *V. Kumar<sup>1</sup>, D. Singh<sup>2</sup>, K. Sperling<sup>3</sup>, J.R. Singh<sup>1</sup>.* 1) Ctr Gen Disorders, Human Gen, Guru Nanak Dev Univ, Punjab, India; 2) Dr. Daljit Singh Eye Hospital, Amritsar, Punjab, India; 3) Institute of Human Genetics, Charite, Humboldt-University, Berlin, Germany.

Purpose: To localize and identify the responsible gene(s) for autosomal dominant congenital cataract (ADCC) in 2 Indian families. Methods: Gene mapping studies using 65 highly polymorphic fluorescently labelled microsatellite markers, selected from known candidate gene regions, were carried out in two large informative ADCC families, showing different clinical types. One showed polymorphic type of cataract while the second had opalescent type of congenital cataract. Genotyping was done on ABI-377 sequencer with GENOTYPER 2.0 software. Two-point and multipoint linkage analyses were performed using the MLINK and LINKMAP components of the linkage program package (ver 5.1) respectively. Results: On basis of the obtained 2-point and multipoint lod score values, we have excluded the known candidate gene regions at 1pter-p36.1, 2q33-35, 3q21-22, 12q13, 13q11.12, 14q24-qter, 15q21-22, 16q22.1, 17p13, 17q24, 19q13.4, 21q22.3, and 22q11.2-12.2 in these 2 families. Conclusion: Our results indicate the existence of at least one more gene underlying autosomal dominant congenital cataract. Since already there exists a report of gene localization for polymorphic congenital cataract at 2q33-35, the exclusion of this locus in present polymorphic type of congenital cataract family supports the existence of genotypic heterogeneity for this type of cataract. In order to achieve better understanding of the phenotype-genotype correlations for the observed heterogeneity in congenital cataract, and for the identification of the various factors involved in lens opacification, the molecular characterisation of different phenotypes is essential.

**Correlation between linkage and association tests in families.** *E.R. Martin, M.P. Bass, E.R. Hauser.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

A common strategy for identifying complex disease genes is to conduct linkage analyses first and then follow significant results with family-based tests for association in an attempt to further localize the disease gene. To help interpret the results from linkage and association tests conducted on the same data, it is desirable to know when the tests are correlated. It has been shown analytically that the transmission/disequilibrium test (TDT), as a test of linkage, and the mean haplotype allele-sharing test of linkage are independent under the null hypothesis of no linkage. However, it is unclear what the relationship is between family-based tests that focus on testing for association and allele-sharing linkage tests. To examine this question, we used computer simulations to compare the pedigree disequilibrium test (PDT), a test for association in pedigree data, and Siblink, a nonparametric affected-sib-pair test of linkage. The PDT tests for deviations from the composite null hypothesis of no linkage or no association, while Siblink tests the null hypothesis of no linkage. Thus it was of interest to examine the correlation between these methods for various levels of both linkage and allelic association. Specifically, we considered a disease locus with a marker locus with: 1) no linkage and no association; 2) linkage and no association; 3) no linkage and association; 4) linkage and association. Model parameters were taken to be consistent with observed SNP data around the APOE locus. Preliminary results suggest that, for complex diseases, substantial correlations are likely to be found only when there is both linkage and allelic association between marker and disease loci. However, as expected, the degree of correlation depends highly on the underlying model. These results will have important implications in how we interpret the joint behavior of linkage tests and family-based tests for association in the same data.

**Sampling strategies for linkage mapping of susceptibility genes in complex diseases with late age of onset using affected sibling linkage methods.** *T. Mary-Huard, M. Martinez.* EMI00-06, INSERM, Evry, France.

For complex diseases, affected sibling (AS) linkage analysis is an alternative to the lod score method. For adult onset diseases, parental genotypes are often unknown, and IBD rates are estimated using the available DNA information in families, under the specified marker allele frequencies. It is clear that the loss of parental DNA information can be balanced by genotyping additional unaffected relatives. Most study designs are based on the analysis of nuclear families with the inclusion of the Unaffected Siblings. For realistic sibship sizes the power of this sampling strategy may remain low. The marker information from other types of unaffected relatives, as the offspring (OF) of the AS, can be worth to include. In this work, we have conducted Monte Carlo simulation studies to compute the power of different family structures: (i) Nuclear Families; AS = 2, US = 0 or 2 and P = 0 or 2 genotyped parents; (ii) 3-Generation Pedigrees: NF (AS = 2, US = 0 or 2 and P = 0) and the OF = 2 for each AS. The power of each sampling strategy was compared as a function of: (1) the type of analysis (i.e., single or multipoint ASP analysis), (2) the Marker Heterozygosity and (3) the Map Density (i.e. 2 to 10 markers spanning a 10cM map). One thousand replicates for each sample size (n) of families were simulated and analysed using two Maximum Likelihood Score methods; MLB [Abel et al 1998] and Zlr [Kong and Cox, 1997] statistics as implemented in the MLBGH and Allegro package programs. Our goal is to propose sampling guidelines for our European genome scan of Parkinsons disease. As expected, there is no advantage in using 3-Generation Pedigrees when using pairwise ASP analyses. The relative power of each ASP statistic is dependent on the genetic model, but MLB and the exponential Zlr statistics are shown to be equivalent in these data. Conversely, when using multipoint ASP analyses, the power of GP tends to be greater than that of NF. The power of the 3-GP strategy increases with the map density, especially when MH is not very low (<60%). The relative power of each strategy is also discussed in terms of amount of marker genotyping effort.

**Comparative genome scans of cleft lip with or without cleft palate in Chinese and Turkish families.** *M.L.*

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Cleft lip with or without cleft palate (CL/P) is a common congenital anomaly, with birth prevalences ranging from 1/500 to 1/1000 depending on the population. Reported birth prevalences are consistently higher in Asian populations than in Caucasians. Therefore, it is of interest to determine whether the CL/P etiological factors in Asian populations differ from those in Caucasians. Families were identified as part of our on-going studies of oral-facial clefts; included in the analyses described here were 36 multiplex families from Shanghai, China and 18 inbred families from Ankara, Turkey. These are the first reported genome-scan studies in either population. Genotyping of Weber Screening Set 9 (387 STRPs with average spacing of ~ 9 cM, range 1-19 cM) was done by the Mammalian Genotyping Service of Marshfield Laboratory. Multipoint LOD scores and multipoint heterogeneity LOD scores (HLODs) were calculated between each marker and CL/P. Calculations were done under a range of assumptions as to the genetic model for CL/P. In addition, the model-free multipoint NPL statistic was calculated. The following regions had positive results (HLOD > 1.0 and/or NPL p-values < 0.05) in the Turkish families: chromosome 1 (168-275 cM), chromosome 3 (26-45 cM), chromosome 4 (33-73 cM), chromosome 6 (34 cM), chromosome 11 (37-85 cM), chromosome 12 (150-166 cM), chromosome 18 (3-41 cM). The following were positive in the Chinese families: chromosome 1 (90-110 cM), chromosome 2 (220-250 cM), chromosome 3 (130-150 cM), chromosome 4 (140-170 cM), chromosome 6 (70-100 cM), chromosome 18 (110 cM), chromosome 21 (30-50 cM). Therefore, a few regions were similar between the two populations, but most of the regions with positive results were unique to either the Chinese or Turkish families. Supported by NIH grants DE09886 and DE12472.



**The Lupus SCOR combined genome scan for systemic lupus erythematosus: common and ethnic-specific susceptibility regions.** *C.D. Langefeld<sup>1</sup>, J. Kelly<sup>2</sup>, K.L. Moser<sup>3</sup>, P.M. Gaffney<sup>3</sup>, R.R. Graham<sup>3</sup>, W.M. Brown<sup>1</sup>, S.S. Rich<sup>1</sup>, J.B. Harley<sup>2</sup>, T.W. Behrens<sup>3</sup>, R.P. Kimberly<sup>4</sup>* for the SCOR in the Genetics of SLE. 1) Wake Forest University School of Medicine, Winston-Salem, NC; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK; 3) University of Minnesota, Minneapolis, MN; 4) University of Alabama, Birmingham, AL.

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease characterized by the production of pathogenic autoantibodies. With a sibling risk ratio near 20, SLE has a strong genetic component. Here, the Specialized Center of Research (SCOR) in the Genetics of SLE, whose goal is to map and clone genes predisposing to SLE and related autoimmune phenotypes, reports the results of a combined genome scan of 313 SLE pedigrees. These pedigrees, containing at least two siblings with SLE, originate from collections assembled at the University of Minnesota (MN: 187 pedigrees, 656 individuals genotyped, 399 SLE patients) and the University of Oklahoma (OK: 126 pedigrees, 681 individuals genotyped, 298 SLE patients). The number of pedigrees (SLE patients) by ethnic group were 226 (499) Caucasian (CA), 57 (133) African American (AA) and 30 (66) other. We constructed a combined map of 703 microsatellite markers (MN: 366, OK: 274, Both: 63) using marker order and distances from the Mammalian Genotyping Center in Marshfield, WI. We report the maximum LOD score (nearest marker, combined LOD, AA pedigrees' LOD, CA pedigrees' LOD) computed from multipoint nonparametric linkage regression analysis using the NPL(pairs) statistic. Three chromosomal regions exceed genome-wide significance: chr 6 (D6S2410, LOD=4.9, AA LOD=1.6, CA LOD=2.6); chr 4 (D4S403, LOD=3.7, AA LOD=0.00, CA LOD=4.5); chr 16 (D16S3253/D16S503, LOD=3.5, AA LOD=0.1, CA LOD=3.2). Additional regions of interest include chr 1 (D1S2785, LOD= 2.1, AA LOD=0.0, CA LOD=1.8); chr 7 (D7S507, LOD= 2.5, AA LOD=0.0, CA LOD=2.9); and chr 20 (D20S481/D20S119, LOD=2.0, AA LOD=0.0, CA LOD=2.7). Among AA pedigrees, the strongest evidence for linkage was 15-20 cM qter of D6S2410 (D6S1031, LOD=3.8, AA LOD=2.5, CA LOD=1.4). Our peak on 1q (D1S2785) coincides with that of Shai et al. (1999).

**Association of Tau with Late-Onset Parkinson Disease.** *S.A. Oliveira<sup>1</sup>, E.R. Martin<sup>1</sup>, W.K. Scott<sup>1,3</sup>, M.A. Nance<sup>3</sup>, R.L. Watts<sup>3</sup>, J.P. Hubble<sup>3</sup>, W.C. Koller<sup>3</sup>, R. Pahwa<sup>3</sup>, M.B. Stern<sup>3</sup>, B.C. Hiner<sup>3</sup>, J. Jankovic<sup>3</sup>, C.G. Goetz<sup>3</sup>, G.W. Small<sup>3</sup>, M.A. Hauser<sup>1</sup>, L.T. Middleton<sup>2,3</sup>, A.D. Roses<sup>2,3</sup>, J.L. Haines<sup>3</sup>, B.L. Scott<sup>1,3</sup>, M.A. Pericak-Vance<sup>1,3</sup>, J.M. Vance<sup>1,3</sup>.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) GlaxoSmithKline, Research Triangle Park, NC; 3) Duke CHG/GlaxoSmithKline PD Genetics Collaboration.

The microtubule-associated protein tau promotes microtubule assembly and stability, and may be involved in the establishment and maintenance of neuronal polarity. Mutations in the tau gene have been identified in several neurodegenerative diseases such as frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), progressive supranuclear palsy (PSP), and pallido-ponto-nigral degeneration (PPND). In a complete genomic screen, we found evidence of linkage in idiopathic Parkinson disease (PD) families to a region on chromosome 17q21 encompassing the tau gene. To investigate whether the tau gene is also involved in the most common form of PD, we tested five single nucleotide polymorphisms (SNPs) within the tau gene for association with PD using family-based tests of association. The SNPs were analyzed individually and in haplotypes. We found evidence of significant association ( $p < 0.05$ ) for three of the five SNPs tested. Furthermore, stronger association was obtained with haplotype analysis, with a positive association with one haplotype ( $p = 0.009$ ) and a negative association with a second haplotype ( $p = 0.007$ ). This study implicates tau as a susceptibility gene for idiopathic PD.

**Chromosome 12 mapping of late-onset Alzheimer disease among Caribbean Hispanics.** *J.H. Lee<sup>1</sup>, R. Mayeux<sup>1,2</sup>, S.N. Romas<sup>1,2</sup>, D. Mayo<sup>3</sup>, V. Santana<sup>1</sup>, J. Williamson<sup>1</sup>, A. Ciappa<sup>2</sup>, H.Z. Rondon<sup>1</sup>, P. Estevez<sup>1</sup>, R. Lantigua<sup>1,2</sup>, M. Medrano<sup>4</sup>, M. Torres<sup>5</sup>, Y. Stern<sup>1,2</sup>, B. Tycko<sup>2</sup>, J.A. Knowles<sup>3</sup>.* 1) Sergievsky Ctr Columbia Univ, NY, NY; 2) Taub Inst Columbia Univ, NY, NY; 3) Columbia Genome Ctr, Columbia Univ, NY, NY; 4) Univ Tecnol de Santiago, DR; 5) Plaza de la Salud Hospit, DR.

Support for linkage to chromosome 12p for late-onset, familial Alzheimers disease (AD) has been inconsistent. We studied 79 families from a study of familial AD among Caribbean Hispanics. We studied 35 markers on chromosome 12, concentrating on regions where previous studies showed evidence for linkage. Two-point linkage analysis based on the affected sibpair model gave the strongest support for D12S1623 (LOD=3.15) and D12S1042 (LOD=1.43). Support for A2M was weak (LOD=0.75). In late onset families (onset>65 yrs), the LOD score for D12S1623 was 1.63, while that for D12S1042 was 1.43. There were too few early onset families. In APOE-e4 negative families (affecteds without 4), the LOD score for D12S1623 was reduced (0.81), while that for D12S1042 was elevated (1.73). The LOD scores for D12S1057 and D12S398 exceeded 1. In APOE-e4 positive families (affecteds with 4), none of the LOD scores reached 1.

Multipoint affected relative pair analysis showed peaks flanking the same markers, D12S1623 (NPL=1.52; p=0.028) and D12S1057 (NPL=1.57; p=0.027). In late-onset families, the NPL score for D12S1623 was 2.01 (0.006), while that for D12S1057 was 1.63 (p=0.021). In APOE-e4 negative families, the region flanking D12S1623 remain elevated (NPL=1.74; p=0.013), but the support for the region D12S1042 decreased (NPL=1.1; p=0.077). In APOE-e4 positive families, there was no evidence of linkage for the region flanking D12S1623, but the NPL score for the D12S1042 region did not change (NPL=1.34; p=0.053).

This study of Caribbean Hispanics with late-onset familial AD provides modest support for linkage to 12p. Further, the evidence of linkage was strongest for D12S1623 in the absence APOE-4, suggesting that this locus may act independently of APOE.

**A familial chordoma locus maps to chromosome 7q33.** X. Yang<sup>1</sup>, M.J. Kelley<sup>2</sup>, J.F. Korczak<sup>3</sup>, E. Sheridan<sup>4</sup>, A.M. Goldstein<sup>1</sup>, D.M. Parry<sup>1</sup>. 1) DCEG, NCI, Bethesda, MD; 2) Department of Medicine, Duke Univ., Durham, NC; 3) Epidemiology Section, Karmanos Cancer Institute and Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI; 4) St. James University Hospital, Leeds, UK.

Chordoma, a rare tumor originating from notochordal remnants, represents 1-4% of all primary bone tumors. Five families with two or more relatives with chordoma have been described worldwide, suggesting the existence of an inherited predisposition. We genotyped 22 family members for 365 STR markers spaced about 10 cM apart and performed a genome-wide analysis for linkage in one reported family with 10 individuals with chordoma. Two-point linkage analysis using the MLINK program from the LINKAGE package was performed under the assumption of autosomal dominant inheritance of a rare disease allele. Possible linkage to chromosomes 4, 7, 17, or 19 was suggested based on two-point lod scores greater than 1.0 in the affecteds-only analysis. Additional STR markers at approximately 2 cM intervals were genotyped in each of these four regions. Chromosome 7q gave the greatest evidence for linkage, with a maximum two-point lod score of 2.21 at  $q=0$  in the affecteds-only analysis. Further analysis of chromosome 7q markers with additional members of this family (total of 11 affected individuals) and two unrelated families with two or three affected individuals, under the assumption of locus homogeneity, showed two-point lod scores greater than 3 at six markers in the affecteds-only analysis ( $Z_{\max}[q=0]=4.05$  at marker D7S500). Multipoint analysis based on only affected individuals gave a maximum lod score of 4.78 using GENEHUNTER with an approximate 2-lod support interval from marker D7S512 to marker D7S684. Haplotype analysis of the three families showed a minimal disease gene region from D7S512 to D7S684, a distance of 11.1 cM and approximately 7.1 MB. These results map a familial chordoma locus to 7q33. Further analysis of this region to identify this gene is ongoing.

**The Silver syndrome variant of hereditary spastic paraplegia maps to 11q12-q14, with evidence for genetic heterogeneity within this subtype.** *H. Patel*<sup>1</sup>, *P.E. Hart*<sup>2</sup>, *T.T. Warner*<sup>2</sup>, *R.S. Houlston*<sup>3</sup>, *M.A. Patton*<sup>1</sup>, *S. Jeffery*<sup>1</sup>, *A.H. Crosby*<sup>1</sup>. 1) Dept Medical Genetics, St George's Hospital Medical School, London, UK; 2) Department of Clinical Neurosciences, Royal Free Hospital School of Medicine, London, UK; 3) Section of Cancer Genetics, Institute of Cancer Research, Surrey, UK.

The hereditary spastic paraplegias (HSPs) are a complex group of neurodegenerative disorders characterised by lower limb spasticity and weakness. Silver syndrome (SS) is a particularly disabling dominantly inherited form of HSP complicated by amyotrophy of the hand muscles. Having excluded the multiple known HSP loci, a genomewide screen for linkage was undertaken in one large multigenerational SS family which revealed evidence for linkage of the SS locus, which we have designated SPG17, to chromosome 11q12-q14. Haplotype construction and analysis of recombination events permitted the minimal interval defining the SPG17 locus to be refined to ~13cM, flanked by markers D11S1765 and D11S4136. A second SS family was not linked to the SPG17 locus demonstrating further genetic heterogeneity in HSP, even within this clinically distinct subgroup. The ciliary neurotrophic factor (CNTF) gene, which comprises just two exons, also resides within this region of the genome. Unfortunately, sequence analysis failed to reveal any disease-specific mutations, excluding this gene from a role in the pathogenesis.

**Strategies for Linkage Analysis of Extended Pedigrees.** *K.A. Rinard<sup>1</sup>, M. Harmon<sup>1</sup>, G. Chan<sup>1</sup>, A. Foti<sup>1</sup>, S. Maruti<sup>1</sup>, A. Parker<sup>1</sup>, R. Martin<sup>1</sup>, J. Meyer<sup>1</sup>, C. Richard<sup>2</sup>, J. Lonquist<sup>3</sup>, T. Partonen<sup>3</sup>, T. Varilo<sup>3</sup>, T. Paunio<sup>3</sup>, J. Ekelund<sup>3</sup>, L. Peltonen<sup>3,4</sup>.* 1) Human Genetics, Millennium Pharmaceuticals, Cambridge, MA; 2) Genetics Institute, Wyeth-Ayerst Research, Cambridge, MA; 3) National Public Health Institute, Helsinki, Finland; 4) Human Genetics, UCLA School of Medicine, Los Angeles, CA.

Large extended pedigrees present unique challenges for linkage analysis. As part of a study of schizophrenia in Finland, we have analyzed an extended pedigree composed of 122 nuclear families. This pedigree extends horizontally and includes many wings connected through clusters of individuals for whom DNA is unavailable. For analysis, we used nonparametric statistics combined with Markov Chain Monte-Carlo methods as implemented in SIMWALK. However, two of these nonparametric statistics we believe to be the most powerful for these data are not usable. The NPLall statistic is computationally infeasible if there are more than 20 affecteds in a pedigree, and statistic B (dominant effect) becomes conservative with such complex pedigree structure. To overcome these issues, we devised a method of breaking the pedigree into smaller pieces to facilitate analysis, by preserving overlapping founder trees when possible and by separating sub-pedigrees connected through untyped individuals. This was done blind to any knowledge of allele sharing. The 122 family pedigree was split into 22 smaller pedigrees containing 437 subjects, 241 with DNA and 91 affecteds. Analysis of these sub-pedigrees produced strong evidence for linkage at chromosome 1q32, with  $p=0.00001$  (NPLall) in the largest sub-pedigree, and weaker evidence in the remaining sub-pedigrees. In comparison, analyses using a large pedigree structure more similar to the actual structure weakens evidence for linkage, with  $p=0.001$  (statistic B). The difference between these two results likely is caused by limitations in the nonparametric statistics, in addition to possible locus heterogeneity. These results highlight the need for improved techniques for linkage analysis.

**A potential locus for Adult-Onset Primary Open Angle Glaucoma (POAG) maps to 2p14-2p16. S.**

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We used 322 affected subjects from 91 families and scanned the entire of genome in search of a new locus for Adult-Onset POAG. Initially, 13 different loci were detected, but additional genotyping did not establish a firm linkage to most of these locations. However, one locus on 2p14-p16 consistently showed linkage in one large British family with 9 affected subjects. This family consists of a total of 113 members, of whom 77 are alive. The phenotype of affected subjects includes low to moderate intraocular pressure, onset after 40 and glaucomatous visual field loss. Forty-nine members representing 3 different generations were sampled and subsequently genotyped for 22 DNA markers. Haplotypes were constructed for 49 affected and normal subjects, ranging from 33-87 years of age. All the 6 living affected individuals (67-83 years of age) share a haplotype from D2S123 to D2S329, a region of approximately 27 cM. However, 3 other subjects with ocular hypertension (OH) have recombined within this region thus limiting this new locus to 8 cM, between D2S123 and D2S2165. Eleven normal subjects with a mean age of 48 years are also gene carriers for at least part of this region. Two-point linkage analysis using only affected, OH and elderly members of this family provided LOD score values ranging from 2.25 to 3.0. Six other British and 1 Jamaican family also share at least smaller portions of this region. When all families were analyzed together, the highest LOD score of 8.84 was obtained for D2S2320. Our results provide preliminary evidence for a new POAG locus on 2p14-2p16 region. Screening of several candidate genes from this region failed to identify any disease-causing mutations.

Supported by: National Eye Institute (EY-09947); UCHC-GCRC (M01RR-06192); International Glaucoma Association; Royal National Institute for the Blind; Bluff Field Charitable Trust, UK.

**Preliminary meta-analysis for BMI in NHLBI FBPP study.** X. Wu<sup>1</sup>, R. Cooper<sup>1</sup>, X. Zhu<sup>1</sup>, A. Weder<sup>2</sup>, I. Borecki<sup>3</sup>, M. Bray<sup>4</sup>, C. Hanis<sup>4</sup>, B. Lewis<sup>5</sup>, N. Risch<sup>6</sup>, P. Savage<sup>7</sup>. 1) Department of Preventive Medicine and Epidemiology, Loyola University Medical Center, Maywood, IL; 2) Division of Hypertension and Hyperlipidemia, University of Michigan, Ann Arbor, MI; 3) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO; 4) Institute for Molecular Medicine and Human Genetics Center, University of Texas Houston Health Science Center, Houston, TX; 5) Department of Epidemiology, University of Alabama, Birmingham, AL; 6) Department of Genetics, Stanford University, Stanford, CA; 7) National Heart, Lung and Blood Institute, Bethesda, MD.

A meta-analysis of genome scans for obesity, characterized as body mass index (BMI), was undertaken using the results from the 8 individual studies conducted separately in the NHLBI Family Blood Pressure Program (FBPP). Data were available on a 6245 individuals from 4 ethnic groups (white, black, Mexican American and Asian); genotyping was on-going for another 5,000 participants. The sample represents by far the largest single collection of genome-wide data scan that has been analyzed for obesity, and provides a test of the reproducibility of linkage analysis for a complex phenotype. Genome-wide linkage analyses were first performed separately in each of the 4 ethnic groups in the four networks using the variance component method. No single region achieved genome-wide significance, nor were results of suggestive significance found in more than one sample. A modified Fishers omnibus procedure was then used to combine the p value across the genome from each scan. The most significant result was found at 30.81 cM on chromosome 7 (LOD = 3.26) from the meta-analysis; the largest LOD score among single studies at this location was 1.88. Other meta-analysis methods based on IBD sharing are being used in on-going analyses. Although admittedly preliminary, these findings suggest that loci/regions with a large and consistent effect on obesity across populations are unlikely to be found with current linkage methods. Under some circumstances, meta-analysis may improve the power detecting linkage in the combined analysis, however, more refined analytic techniques may need to be developed.



**Linkage of congenital fibrosis of the extraocular muscles to chromosome 12 in an Indian family.** *C.P. Venkatesh<sup>1</sup>, V.S. Pillai<sup>1</sup>, A. Raghunath<sup>1</sup>, V.S. Prakash<sup>1</sup>, R. Vathsala<sup>1</sup>, M.A. Pericak-Vance<sup>2</sup>, A. Kumar<sup>3</sup>.* 1) Retinal Ophthalmology, Minto Eye Hospital, Bangalore, Karnataka, India; 2) Center for Human Genetics, Duke University Medical Center, Durham, USA; 3) MRDG, Indian Institute of Science, Bangalore, India.

Congenital fibrosis of the extraocular muscles (CFEOM) is a syndrome of congenital ophthalmoplegia and bilateral ptosis. Classical CFEOM is characterized by congenital nonprogressive bilateral ptosis and external ophthalmoplegia with eyes fixed in a strabismic position. Affected individuals typically must tilt their heads back to compensate for the ptosis and fixed downward position of the globes. CFEOM is genetically heterogeneous disorder. In some families, CFEOM can be inherited as an autosomal recessive trait with a locus mapped on chromosome 11q13 (CFEOM2). CFEOM can also be inherited as an autosomal dominant trait with loci mapped on chromosome 12cen (CFEOM1) in eight families and chromosome 16q24 (CFEOM3) in a single family. We have ascertained a four-generation CFEOM family from the state of Karnataka, India in which the disorder is segregating as an autosomal dominant trait. All the affected individuals of this family have bilateral congenital ptosis and external ophthalmoplegia. The maximum expected lod score in this family (4 allele locus; HET=0.75; 10,000 replicates) is 2.02. To assess linkage in this family to the CFEOM1 (MIM 135700) and CFEOM3 (MIM 604361) loci, microsatellite markers from both within and flanking the candidate regions were genotyped. Linkage to CFEOM3 locus was excluded ( $Z < -2.00$ ), whereas analysis of chromosome 12 markers (pter-D12S61-D12S1631-D12S87-D12S345-D12S59-cen-D12S1048-D12S1668-D12S1090-D12S85-qter) was positive. The maximum two-point lod score was 1.8 at  $\theta = 0$  with marker D12S345. The peak multipoint lod score was 2.36 between D12S87 and D12S345. Markers D12S61, D12S1631, D12S87, D12S345, D12S59, D12S1048 and D12S1668 cosegregated with the disease locus in all affecteds. Recombination in three affecteds between D12S1668 and D12S1090 placed the candidate region centromeric to D12S1090. These data supported linkage to CFEOM1 in this family.

**Chromosome 17 linked dementia in the absence of tau mutations.** *R. Rademakers<sup>1</sup>, M. Cruts<sup>1</sup>, B. Dermaut<sup>1</sup>, G. Roks<sup>2</sup>, M. Van den Broeck<sup>1</sup>, C.M. Van Duijn<sup>1,2</sup>, C. Van Broeckhoven<sup>1</sup>.* 1) Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), University of Antwerp (UIA), Antwerpen, Belgium; 2) Department of Epidemiology and Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands.

We studied a four-generation pedigree with autosomal dominant inherited atypical dementia, containing 13 affected individuals with a mean age at onset of  $63 \pm 7$  years (range: 55 to 78 years). Clinical diagnoses included Alzheimer Dementia (AD), Frontotemporal Dementia (FTD) and Parkinson Dementia (PD). In this family, mutations in the amyloid precursor protein (APP), the presenilins (PSEN) and the microtubule binding domains of the microtubule associated protein tau (MAPT) were previously excluded. To identify the disease-causing gene in this family, a genome-wide search was performed with 400 microsatellite markers defining a 10 cM resolution human index map. Using two-point linkage analysis, we were able to detect conclusive linkage to chromosome 17q21 with a LOD score of 3.18 with marker D17S1868, well above the maximum false positive LOD score of 1.71 obtained in simulation studies. When additional markers were used to refine the candidate region, meiotic recombination events located the disease gene in a 6.4 cM interval between markers D17S946 and D17S958 with a maximum multi-point LOD-score of 5.06 at marker D17S951. Since the MAPT gene, known to cause neurodegeneration in FTD-families, is located within this candidate region, we sequenced all 15 coding or partially coding exons of MAPT for mutations. Also exon -1 and 1 kb sequence of the 5'UTR containing the core- and proximal promoter, as well as intron 13 and at least 60 bp of intronic sequence flanking exon 10 were analyzed. More than 20 sequence variations in non-coding regions, as well as 12 coding sequence variations were identified. However, no sequence variation segregating with the disease haplotype were found. Our findings suggest that either another gene mapping to chromosome 17q21, or a novel mutation in as yet unanalyzed regions of the MAPT gene could be responsible for the atypical dementia segregating in this family.

**Exclusion of genetic linkage to known loci in a large French Canadian family with Lewy body parkinsonism. K.A.**

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Parkinson's disease (PD) is the second most common progressive neurological disorder affecting greater than 3% of the population over age 65 and 10-30 % of cases are familial. The identification of disease causing mutations in families with PD has contributed to the understanding of this complex disorder. We have identified a large French Canadian kindred that segregates Lewy body positive PD as an autosomal dominant trait. This family spans five generations and consists of more than 90 individuals, in which 65 have now been examined. Individuals were genotyped and two point linkage analysis was performed to assess linkage to known PD genes or loci. The  $\alpha$ -synuclein (4q21.3-23), Parkin (6q25.2-27), ubiquitin carboxy-terminal hydrolase L1 (4p16), 4p14-16.3, and 2p13 loci were excluded in this kindred using closely linked markers. The clinical and pathological features of this family are consistent with the diagnosis of Lewy body positive Parkinson's disease. Data from this study further demonstrate genetic heterogeneity for familial parkinsonism and indicate that there is at least one other genetic determinant for Lewy body parkinsonism.

**Evidence of further genetic heterogeneity for both autosomal dominant and autosomal recessive limb-girdle muscular dystrophy.** *A.L. Starling, M. Vainzof, R. Pavanello, M. Canovas, A. Cerqueira, M.R. Passos-Bueno, M. Zatz.* Center for the study of the human genome, IBUSP, University of Sao Paulo, Sao Paulo, Brazil.

Fifteen genes responsible for limb-girdle muscular dystrophy have been identified to date including six autosomal dominant (LGMD1A-F) and nine autosomal recessive (LGMD2A-I). We have ascertained three Brazilian families that were excluded by linkage analysis (and protein studies when possible) for these 15 LGMD causing genes. The first family with nine affected members (7 males and 2 females) in 3 generations displays AD inheritance. Two of the affected patients were submitted to muscle biopsy and electromyography which showed a myopathic pattern in both exams. In all affected members the onset occurred after age 30, with proximal weakness in the upper and lower limbs and a variable presence of calf hypertrophy. Serum creatine kinase (CK) was increased 2 - 9 fold. The second family also with AD inheritance has four affected members (3 males and 1 female) in 2 generations. All of them have a history of cardiac problems, weakness of proximal limbs and a CK increase 3 - 6 fold. The age at onset ranged from 25 to 30 years old. The last family, with four affected males has an atypical inheritance. The affected patients have weakness of proximal limbs (predominantly in the lower limbs in three patients but in the upper limbs in the last one), calf hypertrophy and the age at onset ranged from 10 to 15 years old. The CK was grossly elevated (50-80 fold). Electromyography showed a myopathic pattern and the biopsy showed a normal pattern for dystrophin, calpain, dysferlin, the four sarcoglycans and telethonin. We are currently performing a genome-wide scan to identify the disease loci in these three families. Supported by FAPESP-CEPID, PRONEX and CNPq.

**Complete Genomic Screen in Idiopathic Parkinson Disease.** *W.K. Scott<sup>1,3</sup>, J.M. Stajich<sup>1,3</sup>, B.L. Scott<sup>1,3</sup>, M.A. Nance<sup>3</sup>, R.L. Watts<sup>3</sup>, J.P. Hubble<sup>3</sup>, J.L. Haines<sup>3</sup>, W.C. Koller<sup>3</sup>, R. Pahwa<sup>3</sup>, M.B. Stern<sup>3</sup>, B.C. Hiner<sup>3</sup>, J. Jankovic<sup>3</sup>, F.H. Allen<sup>3</sup>, C.G. Goetz<sup>3</sup>, G.W. Small<sup>3</sup>, F. Mastaglia<sup>3</sup>, L.T. Middleton<sup>2,3</sup>, A.D. Roses<sup>2,3</sup>, J.M. Vance<sup>1,3</sup>, M.A. Pericak-Vance<sup>1,3</sup>.* 1) Duke University Medical Center, Durham, NC; 2) GlaxoSmithKline, Research Triangle Park, NC; 3) Duke CHG/GlaxoSmithKline PD Genetics Collaboration.

The relative contribution of genes versus environment in idiopathic Parkinson disease (PD) is controversial. While genetic studies have identified two genes in which mutations cause rare single gene variants of PD and relative risk studies have suggested a genetic component, recent twin studies have suggested that little genetic contribution exists in the common late-onset forms of PD. To identify genes associated with idiopathic PD, we performed a complete genomic screen (N=344 markers, average spacing 10cM) in 174 multiplex (2 or more sampled affected individuals) idiopathic PD families [containing 870 sampled members (378 affected), 185 sampled affected sibling pairs and 70 other sampled affected relative pairs]. Mean age at onset was  $59.9 \pm 12.6$  years. Diagnosis of PD was based on the presence of two of the following signs: tremor at rest, bradykinesia, and rigidity, as well as the absence of atypical features and other causes of parkinsonism. A clinical adjudication board reviewed all cases for accuracy and consistency across sites. Two-point parametric (MLOD) and multipoint non-parametric (LOD\*) linkage analysis detected significant evidence for linkage (MLOD or LOD\* > 2) to five distinct chromosomal regions: chromosome 6q in the parkin gene in families with at least one individual with PD onset < 40 years; chromosomes 17q, 8p, and 5q in late-onset families, and chromosome 9q in families with evidence of levodopa non-responsive patients. The result on chromosome 17q is in the vicinity of the tau gene. Tau haplotypes are associated with idiopathic PD in our sample (Oliveira et al, this meeting). These data indicate that contrary to previous reports, multiple genetic factors are indeed important contributors to the development of idiopathic late-onset PD.

**Investigation of association of polymorphisms in TNFR1 and TNFR2 in Alzheimers disease patients.** *R. Perry*<sup>1</sup>, *J. Collins*<sup>2</sup>, *L. Harrell*<sup>1</sup>, *D. Blacker*<sup>3</sup>, *R. Tanzi*<sup>3</sup>, *M. McInnis*<sup>4</sup>, *S. Bassett*<sup>4</sup>, *R. Go*<sup>1</sup>. 1) Univ Alabama at Birmingham, Birmingham, AL; 2) Greenwood Genetic Center, Greenwood, SC; 3) Massachusetts General Hospital, Boston, MA; 4) Johns Hopkins University, Baltimore, MD.

Using non-parametric analyses in 266 sib-pair families from the NIMH sibling dataset, we previously reported results of a collaborative genome wide scan where polymorphic markers located at 1p36 and 6p21 were associated with Alzheimers disease (AD) (Collins et al, 2000; Go et al, 1998; Collins et al, 1998). We recently reported a haplotype containing alleles of the 238 and 308 promoter polymorphisms of the tumor necrosis factor (TNF) gene and the microsatellite TNFa, located ~16.5 kb upstream of TNF, was associated with AD in subset of the above families (Collins et al, 2000). From a subset of 320 families with an age of onset over seventy years (total NIMH sibling dataset = 481), we found suggestion of linkage at two regions on chromosome 1, including the same 1p terminal region. The tumor necrosis factor receptor 2 (TNFR2) gene, one of two main receptors for TNF, is located at 1p36.22. We used family-based association testing to look at the T@G polymorphism at position 196 in exon 6 of TNFR2 that leads to a M@R substitution. However, we did not find any significant association between TNFR2 and AD in 150 families with at least two affected and one unaffected siblings (SIBASSOC, p=0.71, S-TDT, p=0.28, SDT, p=0.63). The other receptor TNF binds to, TNFR1, is located at 12 p terminal, a region where other genome wide scans have indicated as suggestively linked to AD (Kehoe et al, 1999; Rogaeva et al, 1998). We genotyped an A@G silent change in codon 12 of exon 1 of TNFR1 and two polymorphisms located in introns 5 and 7 in the same set of families. The codon 12 silent change was significantly associated to AD (FBAT, p=0.02). Preliminary analyses on the other two polymorphisms do not appear to show any significant association with AD. There appears to be significant linkage disequilibrium between the exon 1 polymorphism and the intron 5 polymorphism, but not the intron 7 nucleotide change. Results of further haplotype analyses will be presented at the meeting.

**A score test for detecting quantitative trait loci using sibships of arbitrary sizes.** *K. Wang*<sup>1</sup>, *J. Huang*<sup>2,1</sup>. 1) Dept Biostatistics, Univ Iowa, Iowa City, IA; 2) Dept Statistics and Acturial Science, Univ Iowa, Iowa City, IA.

The Haseman-Elston method is widely used for mapping quantitative trait loci using independent sib-pair data. However, this method does not use all the information in the data because it only considers the sib-pair trait value difference (Wright, 1997). In addition, its generalization to non-independent sib-pairs is not straightforward. Here we introduce a score test statistic derived from a normal likelihood based on multiplex sibship data conditional on IBD sharing probabilities. This score test is asymptotically equivalent to the corresponding likelihood ratio test, but it is easier to implement since it does not require maximization of the likelihood function. Because the proposed test uses all the trait values, not just the pair-wise differences, it makes more efficient use of the data. The proposed test is also naturally applicable to sibships of arbitrary sizes. The performance of the proposed score test are evaluated via simulations. The results indicate that the proposed test is more powerful than the Haseman-Elston method under variety of situations.

**Mapping of Spondylothoracic Dysplasia ( Jarcho-Levin syndrome) to chromosome 2q32.1 in Puerto Rican**

**population.** *A. Santiago-Cornier*<sup>1,3,5</sup>, *N. Ramirez*<sup>4</sup>, *V. Franceschini*<sup>1,3</sup>, *J. Acevedo*<sup>2</sup>, *H. Roman*<sup>1,3</sup>, *E. Rosado*<sup>1</sup>, *L. Garcia*<sup>6</sup>, *J. Torres*<sup>3</sup>. 1) Genetic Division, Ponce School of Medicine, Ponce, PR; 2) Dept. of Nursing, Univ. of Puerto Rico at Arecibo, Arecibo, PR; 3) Dept. of Biochemistry, Ponce School of Medicine, Ponce, P.R; 4) Dept. of Orthopedics, Mayaguez Medical Center, Mayaguez, P.R; 5) Dept. of Pediatrics, Ponce School of Medicine, Ponce, PR; 6) Dept. of Neonatology, University of Puerto Rico School of Medicine, Rio Piedras, PR.

Spondylothoracic dysplasia also know as Jarcho-Levin syndrome (JLS MIM#277300) is an autosomal recessive disorder with high prevalence in Puerto Rican population. JLS main features includes multiple spinal anomalies, fusion of the ribs at the costo-vertebral joint, bilaterally symmetrical fanning out of the chest, umbilical and inguinal hernias and severe restrictive respiratory function. In this investigation we describe the localization of the first Jarcho-Levin syndrome locus and provide evidence that the spondylothoracic dysplasia maps to chromosome 2 in 18 Puerto Rican families. We collected blood samples from 78 individuals, 26 of whom were affected. We performed a genomewide linkage analysis in these families. Haplotype analysis and recombination events localized the disease gene to a 12cM region between markers D2S1384 and D2S1363 with a maximum LOD score of 5.7 and 3.7 respectively for all the informative (11) families. Previous research on a similar disease, spondylocostal dysplasia ,proved to have mutations on Delta homologue, DLL3 gene. These findings provide evidence that both diseases are due to mutations in different genes. Mutation analysis of candidate genes within the region is currently undergoing .Genotype-Phenotype correlations of affected and heterozygous family members will make possible to examine gene dosage in JLS. Evaluation of additional JLS patients will allow us to evaluate a possible founder effect and its extension in Puerto Rican population and to use the narrowest region of shared haplotypes to search for the disease gene.



**The gene for Familial Hypobetalipoproteinemia is linked to a 2.0 cM interval on 3p21.1-22.** *B. Yuan<sup>1</sup>, R.J. Neuman<sup>2</sup>, D.S. Gerhard<sup>3</sup>, P. Yue<sup>1</sup>, K.Y. Liu<sup>2</sup>, S.H. Duan<sup>1</sup>, M. Averna<sup>1</sup>, G. Schonfeld<sup>1</sup>.* 1) Lipid Research, Internal Medicine, Washington Univ, St. Louis, MO; 2) Dept Psychiatry, Washington Univ, St. Louis, MO; 3) Dept Genetics, Washington Univ, St. Louis, MO.

We previously reported linkage of the familial hypobetalipoproteinemia (FHBL) trait to ca.10 cM region on 3p21.1-22 in one kindred with FHBL. We identified and genotyped 21 additional FHBL kindreds (131 individuals and 58 affecteds) to reduce the candidate region and make the identification of the gene feasible. Individuals with abnormally low apoB levels were considered affected, and parametric and nonparametric analyses demonstrated that at least 3 families were potentially linked to the same region as reported previously. Markov chain Monte Carlo linkage analysis of the quantitative apoB level further confirmed linkage to this region. Three recombination events allow us to reduce the candidate region from ca. 10 cM to 2.0 cM, between the markers D3S2407 and D3S1767. To identify the candidate gene, we constructed a 2.2-Mb sequence-ready contig map completely covering the critical region defined by recombination events by assembly of the 56 BAC/PAC clones published in NCBI database. In this region are approximately 200 genes or expressed sequence tags (ESTs). To find the disease gene, we are screening for variations and mutations in the candidate genes using Southern blotting for detection of large-scale genomic rearrangements and direct sequencing to find nucleotide substitutions, microdeletions or insertions.

**Power comparison of phase-known versus phase-unknown haplotype analyses for case-control designs.** *L.E. Green, E.M. Lange, C.D. Langefeld.* Public Health Sciences, Wake Forest University, Winston-Salem, NC.

It is widely known that the relative power of haplotype analyses under a case-control design is greater when haplotype phase is known rather than when phase is probabilistically inferred. We have conducted a simulation study, using likelihood ratio tests, to investigate how many additional cases and controls must be sampled when inferring phase using the EM algorithm in order to have equivalent power to that of 500 cases and 500 controls for which phase is known. We simulated data for a case-control design using a slight modification of the algorithm outlined by Devlin and Risch (1995). We assumed a single biallelic disease-predisposing locus and either 2, 3 or 4 equally spaced biallelic markers (allele frequencies of 0.4 and 0.6). We randomly sampled cases and controls from a simulated population of 95,000 individuals based on a founding population of 500 unrelated individuals and 20 generations of random mating. We varied the recombination fraction ( $q=0.01$  and  $0.001$ ) and the genetic model (dominant, recessive;  $I_s=1.5, 2.0$  and  $3.0$ ) for a total of 12 sets of simulations containing 10,000 replications each. Under each of the conditions, the phase known analysis was more powerful than the phase-unknown analysis for equal sample sizes. For 2, 3, and 4 marker simulations, average increases in sample size of 19%, 42% and 68%, were needed respectively, when inferring phase in order to achieve equivalent power to that of the phase-known analysis. Overall, decreasing  $q$  from 0.01 to 0.001 increased the number of cases and controls needed for the phase-unknown analysis to have equivalent power to that of the phase-known. For example in the 3 marker simulations, decreasing  $q$  required an additional 57 cases and controls for equivalent power. Varying the genetic model (i.e.,  $I_s$ , dominant and recessive models) did not appear to qualitatively alter the above conclusions. These results suggest that, conditional on the specific costs, it may be more effective to recruit additional cases and controls and infer phase than to determine exact phase via recruiting additional family members or through laboratory techniques.

**Genetic heterogeneity in hemifacial microsomia: evidence for three loci at 14q32, 2q35 and 11q12-q13. D.**

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Hemifacial microsomia (HFM) is a common birth defect involving first and second branchial arch derivatives. The phenotype is extremely variable, in addition to craniofacial anomalies there may be cardiac, vertebral and central nervous system defects. The majority of cases are sporadic, but there is substantial evidence for genetic involvement in this condition including rare familial cases that exhibit either autosomal dominant or recessive inheritance. A genome wide search for linkage in three families with features of HFM (two dominant, one recessive) was performed to identify the disease loci. Linkage was identified, in one family, to a region of approximately 10.7cM on chromosome 14q32, with a maximum multipoint lod score of 3.00 between microsatellite markers D14S987 and D14S65. This locus harbours the Goosecoid gene, an excellent candidate for HFM based on mouse expression and phenotype data. Coding region mutations and gross rearrangements of the gene were excluded. No exonic variation was observed in 120 sporadic cases of HFM. Evidence for genetic heterogeneity is provided by the second family in which linkage was excluded from this region. Linkage analysis in this family was suggestive of a disease locus on chromosome 11q12-q13. A locus at 2q35 has been found in a recessive family with HFM associated with external auditory canal atresia, deafness, acro-osteolysis and Mullerian anomalies. This region harbours two members of the WNT protein family.

**Modelling admixture and extracting information about linkage that is generated by admixture from marker genotypes.** *C.J. Hoggart<sup>1</sup>, P.M. Mckeigue<sup>1</sup>, D. Clayton<sup>2</sup>.* 1) London School of Hygiene and Tropical Medicine, London, UK; 2) Department of Medical Genetics, Cambridge University, UK.

Where there has been admixture between two or more ethnic groups that vary in disease risk for genetic reasons, it is possible to map the genes underlying this ethnic variation in a manner analogous to linkage analysis of an experimental cross. This approach relies on selection of markers that have large frequency differentials between the parental populations. We report preliminary results with a statistical analysis program which models admixture using a Bayesian approach, giving the posterior distribution of individual admixture and ancestry at each locus by Markov chain Monte Carlo simulation. The program can deal with any number of parental populations and any number of linked marker loci. Information about the ancestry of one or both parents can be included where available. We describe several applications of this program to data on admixed African-American and Caribbean populations. In case-control and cross-sectional studies, we have modelled the relationship of individual admixture to risk of diseases such as hypertension and systemic lupus erythematosus. In case-only or cross-sectional studies, we can test each marker locus for linkage with genes that underlie these ethnic differences in disease risk. We can also test whether the ancestry-specific allele frequencies (probabilities of each allelic state given the ancestry of the allele) have been mis-specified. Where necessary we can re-estimate these frequencies for each admixed population under study. This makes it possible to apply admixture mapping even where allele frequencies in one or both parental populations are unknown.

**Use of microcell mediated mouse chromosome transfer for gene localization.** *A.N. Mankad<sup>1</sup>, M.P. Cosma<sup>2</sup>, B.A. Cox<sup>1</sup>, C. Reifsteck<sup>1</sup>, S. Olson<sup>1</sup>, M. Grompe<sup>1</sup>.* 1) Molecular and Medical Genetics, Oregon Health & Science Univ., Portland, OR; 2) Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy.

Microcell mediated chromosome transfer, a recognized technique for introduction of a single chromosome into recipient cells, combined with an assay for complementation of a cellular genetic defect, can be used to localize a gene to a particular chromosome. The standard microcell donors contain single, specific human chromosomes in a mouse background. Human chromosome transfer using these donors unambiguously identifies the complementing chromosome; however, as many as 22 MMCT fusions may be required. In addition, sub-localization of the gene relies on polymorphic differences between donor and recipient chromosomes, which may be difficult to find in the region of interest. One way to circumvent these difficulties is to transfer mouse chromosomes rather than human ones, and assay for complementation of the genetic defect by a mouse gene. The donor cells for this modified MMCT are mouse A9 fibroblasts that have been infected with retrovirus carrying a neomycin resistance cassette, which integrates randomly, marking approximately one chromosome per cell. Since a different neo-marked mouse chromosome is transferred from each donor cell, each fusion potentially covers the entire genome, not just a single chromosome. Additionally, pervasive species differences make it easy to assay for successful mouse chromosome transfer, as well as sub-localize or map the mouse gene, via mouse chromosome FISH, mouse microsatellite PCR, or B2 repeat PCR. The latter amplifies DNA between ubiquitous rodent-specific repeat sequences with a single primer, and the product appears as a smear with a distinct series of bands for every possible subset of chromosomes. Six microcell fusions applying the modified protocol generated 54 hybrid clones from one recipient cell line; of these, about 30 were independent clones, only one was a whole cell hybrid, and one was a confirmed mono-chromosomal hybrid. These results validate the use of this microcell mediated mouse chromosome transfer technique for rapid generation of independent hybrids and scanning of the genome for localization of a gene of interest.

**Association Studies for Quantitative Traits in Structured Populations.** *K.M. Roeder<sup>1</sup>, S-A. Bacanu<sup>2</sup>, B. Devlin<sup>2</sup>.* 1) Dept Statistics, Carnegie Mellon Univ, Pittsburgh, PA; 2) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA.

Association between disease and genetic polymorphisms often contributes critical information in our search for the genetic components of common diseases. During 1999, Devlin and Roeder (*Biometrics* 55:997-1004) introduced genomic control, a statistical method that overcomes a drawback to the use population-based samples for tests of association, namely spurious associations induced by population structure. In essence, genomic control (GC) uses markers throughout the genome to adjust for any inflation in test statistics due to substructure. To date genomic control (GC) has been developed for binary traits and bi- or multiallelic markers. Tests of association using GC have been limited to single genes. In this report, we generalize GC to quantitative traits (QT) and multilocus models. Using statistical analysis and simulations, we show that GC controls spurious associations in reasonable settings of population substructure for QT models, including gene-gene interaction. Through simulations we explore GC power for both random and selected samples, assuming the QT locus tested is causal and its specific heritability is 2.5 - 5%. We find that GC, combined with either random or selected samples, has good power in this setting, and that more complex models induce smaller GC corrections. The latter suggests greater power can be achieved by specifying more complex genetic models, but this observation only follows when such models are largely correct and specified a priori.

**Outlier Detection and False Discovery Rates for Whole-genome Association Studies.** *J-Y. Tzeng<sup>1</sup>, B. Devlin<sup>2</sup>, K.M. Roeder<sup>1</sup>, L. Wasserman<sup>1</sup>.* 1) Dept Statistics, Carnegie Mellon University, Pittsburgh, PA; 2) Dept Psychiatry, Univ Pittsburgh, Pittsburgh, PA.

Benjamini and Hochberg (J. R. Stat. Soc. Ser. B, 1995, 57:289-300) introduced an approach to multiple hypothesis testing that controls the false discovery rate (FDR), defined as the fraction of false rejections among those hypotheses rejected. The procedure is very appealing because it controls a quantity that is often of greater scientific relevance than the overall type I error rate. When applied to a genome scan it chooses a near optimal threshold value that balances costs of both false non-discoveries and false discoveries. We define a statistic, called the matching statistic, for locating regions of the genome that exhibit excess similarity among cases when compared to controls. Such regions are reasonable candidates for harboring disease genes. We find the asymptotic distribution of the statistic while accounting for correlations among sampled individuals. We use the FDR method for multiple hypothesis testing to find regions of excess sharing. The p-values for each region involve estimated nuisance parameters. We show that the FDR method based on p-values and with estimated nuisance parameters asymptotically preserves the FDR property.

**Fine mapping on chromosome 20 shows evidence of linkage for BMI and fat mass in type 2 diabetes affected sib-pairs from West Africa.** C. Rotimi<sup>1</sup>, R. Kittles<sup>1</sup>, D. Parish-Gause<sup>1</sup>, G. Chen<sup>1</sup>, G. Vosgianian<sup>1</sup>, P. Furbert-Harris<sup>1</sup>, A. Amoah<sup>2</sup>, J. Acheampong<sup>3</sup>, J. Oli<sup>4</sup>, B. Osotimehin<sup>5</sup>, T. Johnson<sup>6</sup>, G. Dunston<sup>1</sup>, F. Collins<sup>7</sup> and AADM Study Investigative Group. 1) National Human Genome Center, Howard University, Washington, DC; 2) University of Ghana; 3) University of Science and Technology, Ghana; 4) University of Nigeria Teaching Hospital; 5) University College Hospital, Nigeria; 6) University of Lagos, Lagos, Nigeria; 7) National Human Genome Research Institute, Bethesda, MD.

Several candidate genes for type 2 diabetes and associated risk factors have been mapped to chromosome 20 (chr20). We performed fine mapping of two regions spanning 66 cM at 20p11.2-20p13 and 20q12.3-20q13.1. These regions were mapped using 13 highly polymorphic microsatellite markers in a newly identified cohort of 360 affected sibling pairs (ASP) with type 2 diabetes, located in two countries in West Africa. Equal numbers of patients were enrolled from three sites in Nigeria (Enugu, Ibadan and Lagos) and two sites from Ghana (Accra and Kumasi). Populations of the west coast of Africa, from Senegal to Angola, were major contributors of Africans to the Americas and other parts of the world during the Middle Passage. By studying West Africans we hope to shed light into reasons for the apparent increased susceptibility of African Americans to diabetes, obesity and associated complications. Using affected sib-pair analysis as described in GENEHUNTER and S.A.G.E (sibpal2), we obtained suggestive evidence of linkage between BMI, fat mass and a 17cM region (31.2-48.5) on chr20. The strongest evidence occurred at the position corresponding to marker D20S471 (location 20p11.22) with a maximum logarithm of odds score (MLS) of 2.3 for BMI and 2.5 for Fat mass. The single-point analysis p-value was 0.0294 for BMI and 0.0279 for fat mass. Our findings suggest that one or more genes affecting obesity may be located in 20p11.22 region of chr20. Since this chromosomal region includes several plausible candidate genes for obesity, we suggest that more detailed analyses are needed to elucidate the putative gene(s) for obesity and related phenotypes in this and other populations.



**The low activity H1 haplotype of *PDGFRA* P1 promoter may predispose to spina bifida in individuals of Mexican descent.** K.-S. Au<sup>1</sup>, K.A. Volcik<sup>1</sup>, S. Featherston<sup>1</sup>, H. Northrup<sup>1,2</sup>. 1) Dept Pediatrics, Univ Texas Medical Sch, Houston, TX; 2) Shriners Hospital for Children, Houston, TX.

Abnormal platelet-derived growth factor alpha-receptor (*Pdgfra*) expression has been suspected to cause spina bifida (SB) in various mouse models. *PDGFRA* binds to both homodimers and heterodimers of PDGF-A and PDGF-B whereas PDGF b-receptor binds only to PDGF-B. It was postulated that either premature- or delayed- differentiation of neural glia and cartilage precursor cells could result in abnormal closure of neural tube. Various isoforms of human *PDGFRA* were identified and two separate promoters (P1 and P2) control their expression. Promoter P1 is located 5' to exon 1 and P2 is located in intron 12 of *PDGFRA*. Retinoic acid treatment stimulates *PDGFRA* transcription from P1 and promotes differentiation of Tera-2 cells, while expression from P2 promotes proliferation of the Tera-2 cells. Eight SNPs in the P1 region were identified and found to contribute to 5 different haplotypes (Joosten et al., 2001). Two major haplotypes, H1 and H2a, were detected in about 90% of those studied. The H1 haplotype consists of nucleotide changes that interrupt the SP-1 and GCF binding sites. Promoters with the H1 haplotype have only 1/6 the activity of those with the H2a haplotype. Presence of the H1/H2a haplotype was suggested to increase the risk for NTDs. We examined the *PDGFRA* promoter in a group of 85 sporadic SB patients of Mexican descent and found over-representation (49.4%) of the H1/H2a haplotype. Interestingly, 20% of our patients were homozygous for the H1/H1 haplotype. Together, homozygous and heterozygous H1 haplotypes constituted 69.4% of our SB population. Our finding is consistent with the double mutant patch/undulated mouse model suggesting that low activity of the *PDGFRA* predisposes to spina bifida. Low expression of the P1-derived mRNAs controlled by H1 haplotype in SB patients may promote expression of *PDGFRA* isoforms from P2 by default and delay differentiation of neural glia and cartilage precursor cells for proper closure of neural tube. A larger sample (450 SB patients and 700 unaffected parents) is currently undergoing testing. (Joosten et al., Nat.Genet.27:215-7,2001).

**A high-throughput SNP typing system for genome-wide association studies.** *Y. Ohnishi<sup>1</sup>, T. Tanaka<sup>1</sup>, K. Ozaki<sup>1</sup>, R. Yamada<sup>1</sup>, A. Sekine<sup>1</sup>, H. Suzuki<sup>2</sup>, Y. Nakamura<sup>1,2</sup>.* 1) SNP Research Center, RIKEN, Tokyo, Japan; 2) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

One of the most difficult issues to be solved for the whole-genome association study using SNPs is reduction of the amount of genomic DNA for genotyping. To genotype an individual blood sample for hundreds or thousands of SNPs by any of the presently-available technologies requires too large quantity of genomic DNA to be practical. To overcome this problem, we combined the Invader assay system with multiplex PCR carried out in the presence of antibody to Taq polymerase as well as a novel 384-well card system that can reduce a reaction volume very small. We amplified 100 genomic DNA fragments, each containing one SNP, in a single tube, and analyzed each SNP by the Invader assay. This procedure correctly genotyped 98 of the 100 SNP loci examined, in PCR-amplified samples from 10 individuals; the genotypes were confirmed by direct sequencing. The reproducibility and universality of the method were confirmed using two additional sets of 100 SNPs in 100 genomic fragments. Since we used 40 nanogram of genomic DNA as a template for multiplex PCR, the amount needed to assay one SNP was only 0.4 nanogram; therefore more than 200,000 SNPs theoretically could be genotyped at once when 100 microgram of genomic DNA is available. Our results strongly indicate the feasibility of undertaking genome-wide association studies using blood samples of only 5-10 milliliter. We have constructed a high-throughput genotyping facility using these technologies, which allows us to perform as many as 384,000 typings in one day.

**Haplotype analysis of the IL-1b gene and positive correlation with extracellular IL-1 beta protein levels.** *S.K. Hall<sup>1</sup>, D.G Perregaux<sup>3</sup>, C.A Gabel<sup>3</sup>, L.K. Durham<sup>2</sup>, A.B. Seymour<sup>1</sup>*. 1) Pharmacogenomics, Pfizer, Groton, CT; 2) Biostatistics and Reporting, Pfizer, Groton, CT; 3) Inflammation, Pfizer, Groton, CT.

The cytokine Interleukin (IL)-1 is a primary mediator in the pathogenesis of inflammatory diseases such as rheumatoid arthritis (RA), asthma and inflammatory bowel disease (IBD). Genetic variation within the IL-1 gene cluster has been associated with increased risk to develop these diseases. Previous studies have associated a C3953 T variant in exon 5 of IL-1b with increased LPS-stimulated IL-1b protein secretion as well as linking two promoter variants to an increased risk of H. pylori induced hypochloridia and gastric cancer. We have investigated these polymorphisms as a haplotype within the IL-1b gene to test for a genetic contribution to interpatient variance observed with a blood-borne, two-step cytokine production assay that measures extracellular IL-1b levels generated in response to LPS and the combination of LPS/ATP; the nucleotide triphosphate enhances posttranslational processing. LPS-stimulated blood samples from 31 healthy donors were assessed for extracellular IL-1b. Genotypes were determined at three polymorphic loci throughout the IL-1b gene: C-511T, T-31C, and C3953T in each of the 31 subjects to test for association with secreted IL-1b protein levels. We observed very strong linkage disequilibrium between the -511 and -31 polymorphisms ( $p < .000000000005$ ), in particular between the T allele at -511 and the C at -31. Individuals homozygous for the haplotype comprised of T-511, C-31 and C3953 (T-C-C) had a significantly higher mean level of LPS-induced IL-1b secretion compared to individuals with zero or one copy of this haplotype (3.5 ng/ml vs 1.7 ng/ml,  $p = .036$ ). These data provide suggestive evidence that genetic variation within regulatory elements of the IL-1b gene may be associated with altered posttranslational processing efficiency.

**Determination of single nucleotide polymorphisms using a high throughput mass spectrometry based system. B. Darnhofer, M. Denissenko, L. Leppin, K. Smylie, C. Rodi. Molecular Biology, SEQUENOM, Inc., San Diego, CA.**

Detailed mapping of single nucleotide polymorphisms (SNPs) in the human genome is believed to greatly assist in determining the origins of complex diseases and understanding the causes of adverse drug reactions. Validating the diagnostic candidate SNPs can be a challenging task because the frequency of the contributing polymorphisms is often very low. Furthermore, the genetic basis of many diseases is often composed of not one, but multiple gene variants. To facilitate the scoring of SNPs, the rapid high-throughput MassARRAY system has been developed, which provides a fully automated and accurate solution to these challenges. This system is complemented by a single-tube assay method in which the genotyping primer is annealed to the amplified target in a region immediately adjacent to the polymorphic site. The primer is then enzymatically extended a few bases through the polymorphic site and terminated. The resulting diagnostic product is then robotically dispensed onto a chip and the genotype automatically determined using the mass obtained with MALDI-TOF (Matrix Assisted Laser Desorption Ionization - Time Of Flight) mass spectrometry. This method is fully compatible with 96 as well as 384 microtitre plate formats and further increases in throughput can be realized through the use of multiplexing. Multiplexed analysis data for 192 SNP-containing sequences are presented.

**Randomly assembled arrays for SNP genotyping.** *M. Chee<sup>1</sup>, J.-B. Fan<sup>1</sup>, M. Wenz<sup>2</sup>, E. Wickham<sup>1</sup>, K. Hayashibara<sup>2</sup>, J. Chen<sup>1</sup>, T. Paner<sup>2</sup>, D. Doucet<sup>1</sup>, L. Zhou<sup>1</sup>, B. Kermani<sup>1</sup>, R. Shen<sup>1</sup>, M. Hansen<sup>1</sup>, F. Steemers<sup>1</sup>, C. Zhao<sup>1</sup>, S. Barnard<sup>1</sup>, D. Che<sup>1</sup>, K. Gunderson<sup>1</sup>, D. Barker<sup>1</sup>, J. Efcavitch<sup>2</sup>, A. Oliphant<sup>1</sup>.* 1) Illumina, Inc., San Diego, CA; 2) Applied Biosystems, Foster City, CA.

Randomly ordered, self-assembled arrays of beads provide a novel and versatile technology platform for intrinsically parallel analysis of complex biological samples. Arrays of beads are randomly assembled onto patterned optical fiber bundles (1). Individual beads have diameters of ~ 3 microns, and ~ 50,000 beads are assembled into a single array. Each bead contains oligonucleotide probes which hybridize with high specificity to complementary sequences in a complex nucleic acid mixture. The identity of each bead, unknown at the time of array assembly, is determined by a hybridization-based decoding procedure.

We have developed a highly multiplexed method for SNP genotyping by combining an oligonucleotide ligation-based assay with read-out on random arrays of universal capture probes (2). In addition, by formatting the miniaturized arrays into a matrix that matches a 96-well microtiter plate, many samples can be processed simultaneously and efficiently in an automated fashion. This combination of technologies provides a system for genetic analysis with the capacity and versatility to match the needs of analysis on a genomic scale. Fundamental aspects of the technology will be reviewed, and the application of these arrays to large-scale SNP genotyping will be discussed.

(1) Michael et al. (1998) *Analytical Chemistry* 70, 1242-1248.

(2) Gerry et al. (1999) *Journal of Molecular Biology* 292, 251-262.

**Novel multiplex PCR device used for CA-repeat and SNP genotyping.** *E.S. Mansfield<sup>1</sup>, J. Wu<sup>1</sup>, B. Chen<sup>1</sup>, A. Nguyen<sup>1</sup>, D. Dutta<sup>1</sup>, M. Pho<sup>1</sup>, G. Bogdan<sup>1</sup>, D. Albagli<sup>1</sup>, M.T. Cronin<sup>1</sup>, P. Fortina<sup>2</sup>, R. Anderson<sup>1</sup>.* 1) ACLARA BioScience, 1288 Pear Ave. Mountain View, CA 94043; 2) The Children's Hospital of Philadelphia, 310C Abramson Building, 34th and Civic Center Blvd. Philadelphia, PA 19104.

Mapping of Mendelian and complex genetic disorders has been facilitated by availability of dense genetic maps of common sequence polymorphisms. CA-repeat genotyping has successfully confirmed linkage of a susceptibility locus (IBD1) on chromosome 16 for Crohn's disease, a common complex genetic disorder (AJHG 68: 1165-1171, 2001) and led to the identification of the gene by directing both genetic and physical mapping projects (Nature 411: 599-606, 2001). Generally simple sequence repeat markers such as CA-repeats are analyzed as pooled products from multiple single-plex PCR reactions to prevent primer interaction, allelic dropout or appearance of excessive peak suttering that complicate analysis or may lead to typing errors. This process is inefficient since it adds both sample processing steps and causes substantial reagent waste. We describe the use of a novel LabCard device under development at ACLARA to co-amplify a set of 10 CA-repeat markers linked to the IBD1 gene region. More consistent representation of allelic products and better signal-to-noise were observed when the multiplex PCR was carried out in the prototype LabCard device than conventional multiplex tube reactions. Further, the device enables 960 PCR reactions to be simultaneously carried out in industry standard 96-well thermal cyclers. Representative analytical data from the MegaBACE and ABI 3100 capillary array systems will be presented. We have also used prototype devices for multiplex target amplification in primer extension-based SNP genotyping assays.

**SNP-TRAP, a simplified method for detecting SNPs.** *A. Orpana*<sup>1</sup>, *P. Pajukanta*<sup>2</sup>, *A. Palotie*<sup>3</sup>, *L. Peltonen*<sup>2</sup>. 1) Clinical Chem. and Med. Genet., Univ. of Helsinki, Helsinki, Finland; 2) Dept. of Human Genetics and; 3) Dept. of Pathology, UCLA, Los Angeles, Ca.

The production of sufficient amounts of SNP data for genetic analyses of large study samples requires high specificity, sensitivity and accuracy from the analytical method applied. A number of SNP analysis techniques including both solid phase (e.g. arrays) technologies and homogenous assays, as well as electrophoresis-based techniques, have been introduced to accommodate genotyping needs for increasing numbers of SNPs available. In addition to relative complexity of current methods, the high assay and instrumentation costs have become increasingly important concerns. Labelling or modification of oligonucleotide primers with anchoring groups, together with mandatory purification steps, increases the cost, becoming a challenge for high volume genotyping. We have developed a robust and easily applicable assay without the need to purchase kits or expensive special equipment. The SNP-TRAP-method uses standard primers, basic DNA polymerase and a commercial fluorescence dye. The SNP is detected by cyclic extension of a primer in the presence of one of the biotinylated deoxyribonucleotides, trapping of the biotin-dNTP-labelled primer onto streptavidin plates and quantitating the bound oligonucleotide using a single stranded DNA specific fluorescent dye. We employed the SNP-TRAP in the fine mapping of 33 intragenic SNPs in chromosome 1q21, linked to familial combined hyperlipidemia. The SNPs were selected using public and commercial databases, and the primers were designed with the help of several primer design programs available in the web. The majority of the SNPs (75%) resided in the 5 and 3 regions of the genes, often containing analytically challenging DNA structures e.g. long GC stretches and repeats. The unequivocal genotype calling was successful for 70% of SNPs: out of 47 SNPs, 33 were successfully genotyped on the first round. All SNPs were confirmed by an independent method. For SNP-TRP genotyping we developed an allele calling algorithm facilitating easy data production. The reliability and ease of SNP-TRAP provides a new tool for low- to mid-scale SNP screening.

**Phenotype stratification of linkage analysis in primary open-angle glaucoma (POAG): Evidence for age of onset heterogeneity.** *R.R. Allingham<sup>1</sup>, J.L. Wiggs<sup>2</sup>, K.R. LaRocque<sup>1</sup>, M.A. Hauser<sup>1</sup>, F.L. Graham<sup>1</sup>, B. Broome<sup>1</sup>, E.A. del Bono<sup>2</sup>, R. Bailey<sup>3</sup>, J.L. Haines<sup>3</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Duke Univ Medical Ctr, Durham, NC; 2) Massachusetts Eye and Ear Infirmary, Boston, MA; 3) Vanderbilt University, Nashville, TN.

We employed phenotype stratification to further examine previously identified chromosomal regions of interest in POAG. Families (n = 86) with at least 2 affected members were analyzed. POAG was defined as: age of onset (AOO) (based on age of first diagnosis (AOD)) > 35yrs and 2 of the following criteria: IOP > 21 mmHg both eyes, glaucomatous optic neuropathy both eyes, and visual field (VF) loss. Families were stratified using the following criteria based on the clinical features of one affected individual: AOD < 45 yr; IOP > 29 both eyes; severe VF loss or glaucoma-related blindness both eyes; or history of glaucoma surgery. Combinations of these risk factors were also analyzed. Linkage analysis of phenotypically stratified family sets was performed on regions on chromosomes 1,4,14,15,17, and 19, identified in a genome-wide scan (Wiggs et al, 2000). Linkage analysis was performed using model-dependent (MLOD) and model-independent (MLS) methods. Stratification of POAG families by IOP, VF loss, history of glaucoma surgery, or combinations of these criteria did not affect the previous linkage results. However, stratification by AOO alone gave increased evidence for linkage to the chromosome 15 candidate region increasing linkage results in 4 adjacent markers (GABRB3, D15S822, D15S217, D15S165) with MLOD and/or MLS scores > 1.5. Maximal MLS and MLOD scores were 2.43 (at D15S217) and 2.32 (at D15S822), respectively. These data provide evidence for genetic heterogeneity based on AOD in families with POAG. Monte Carlo simulation methods will be used to confirm the significance of these findings. Similar findings have been described in other late-onset disorders such as Alzheimer disease and breast cancer. These data suggest that AOO is a potentially useful stratification variable in identifying genes in POAG. Support: The Glaucoma Research Foundation, Barkhouser Glaucoma Research Fund, and NIH Grant EY10886.



**Trp64Arg mutation in the 3-adrenergic receptor gene can predict difficulty in improving body fat distribution, glycemic control and insulin resistance in response to hypocaloric diets in overweight male patients with cardiovascular disease.** *H. Ryu<sup>2</sup>, E.Y. Cho<sup>1</sup>, J.H. Kwon<sup>1</sup>, E.K. Im<sup>1</sup>, Y.S. Jang<sup>1</sup>, O.Y. Kim<sup>2</sup>, J.H. Lee<sup>1,2</sup>, H.Y. Park<sup>1</sup>.* 1) Cardiovascular Genome Center, Seoul, South Korea; 2) Dept of Food and Nutrition.

The purpose of this study was to determine whether Trp64Arg mutation in the 3-adrenergic receptor gene affects the ability of overweight cardiovascular disease (CVD) men to improve body fat distribution, glycemic control and insulin resistance in response to hypocaloric diets. Twenty seven overweight heterozygous CVD men with the genetic variant were compared with 55 homozygous overweight CVD men with the normal allele. There were no significant differences in baseline characteristics such as age, BMI, serum concentrations of lipids, glucose and insulin between the 2 groups. Both groups followed the hypocaloric diet calculated from the total caloric requirement minus 300 kilocalories for 16 weeks. Body weight decreased by 4% ( $P < 0.001$ ) in the normal allele group and 3% ( $P < 0.001$ ) in the Trp64Arg variant group. CVD males with the normal allele showed a decrease in the serum concentrations of triglyceride (-20%), total cholesterol (-7%), LDL cholesterol (-7%) and apolipoprotein B (-8%) and 4-11% decrease in total and visceral fat area at L1 and L4 levels after the weight loss. A weight loss in CVD men without the mutation decreased fasting levels of insulin (-24%), the area under the curve (AUC) for glucose (-11%) and insulin (-23%) and increased the AUC for C-peptide (+35%) during oral glucose tolerance test. CVD men with the Trp64Arg variant showed no significant changes in serum lipids, glucose and insulin levels and visceral fat area, even though calorie intake, physical activity levels and baseline characteristics were similar in both groups. These present results show that the Trp64Arg variant of the 3-adrenergic receptor gene may predict difficulty in improving serum lipid profiles, glycemic control, insulin resistance and visceral fat accumulation in response to hypocaloric diets in overweight patients with CVD.

***CD18, A Positional Candidate Influencing Eosinophilia in the Hutterites. J.M. PINTO<sup>1</sup>, M. ABNEY<sup>1,2</sup>, M.S. MCPEEK<sup>1,2</sup>, D.H. SCHNEIDER<sup>1</sup>, D.K. NOLAN<sup>1</sup>, R. PARRY<sup>4</sup>, A.I. SPERLING<sup>3</sup>, C. OBER<sup>1</sup>.*** 1) THE DEPARTMENTS OF HUMAN GENETICS; 2) STATISTICS; 3) MEDICINE, THE UNIV. OF CHICAGO, CHICAGO, IL 60637; 4) THE DEPARTMENT OF MEDICINE, THE UNIV. OF SOUTH DAKOTA, SIOUX FALLS, SD 57105.

Eosinophilia is a phenotype associated with asthma and atopy. A genome-wide screen for loci influencing the quantitative trait eosinophilia was conducted in the Hutterites, a founder population of European ancestry. This population has several advantages for mapping studies, including extensive linkage disequilibrium (LD), fewer susceptibility alleles, and a communal lifestyle ensuring similar environment. Peripheral blood eosinophils were measured in 580 subjects who are derived from 64 founders and are members of a 1,623-person pedigree. A genome-wide screen identified a linkage to D21S1446 ( $P=0.00018$ ) by homozygosity-by-descent (HBD) mapping, a novel method for mapping QTLs in large inbred pedigrees. In this approach, the relatedness among subjects is accounted for by variance components (Abney et al., 2000, *Am J Hum Genet* 66:629). The b2 integrin chain (*CD18*) locus is 3.91 cM from D21S1446. This gene encodes the b subunit of several leukocyte cell adhesion molecules, including LFA-1. *CD18* has been implicated in antigen induced eosinophil recruitment and Th2 cell homing in animal models of atopic disease and also in eosinophil degranulation *in vitro*. It is thus a promising candidate for allergic disease. Using public database searches and DHPLC we identified three variants in *CD18* in the Hutterites: 3'UTR (C/T), intron 8 (C/T), and exon 11 (A/G). All individuals were genotyped for these variants. Using the HBD method, homozygosity for the 3'UTR C allele was significantly associated with lower eosinophil levels ( $P=0.0027$ ). Neither the other variants nor haplotypes showed an association with this phenotype. These results suggest a direct role for the 3'UTR variant or a variant in LD with the 3'UTR C allele in influencing eosinophil levels in humans. Supported by NIH grants 5-T32-DC00058 and HL-56399.

**Mapping the gene for Noonan syndrome (NSI) to a 200-kb region on chromosome 12q24.2.** O. Bartsch<sup>1</sup>, A. Frensel<sup>1</sup>, D. Novotna<sup>2</sup>, E. Kocarek<sup>2</sup>, V. Krutilkova<sup>2</sup>, M. Havlovicova<sup>2</sup>, P. Goetz<sup>2</sup>, G. te Kronnie<sup>3</sup>, E. Pantchechnikova<sup>4</sup>, L. Musante<sup>4</sup>, H.H. Ropers<sup>4</sup>, V. Kalscheuer<sup>4</sup>. 1) Institut für Klinische Genetik, Technische Universität Dresden, Germany; 2) Inst. of Biology and Medical Genetics, 2nd Medical Faculty, Charles University, Prague, Czech Republic; 3) Dept. of Pediatrics, Lab of Oncology, University of Padova, Italy; 4) Max-Planck-Institute for Molecular Genetics, Berlin, Germany.

Noonan syndrome (NS) is a well known autosomal dominant disorder. Jamieson et al. (Nature Genetics 1994;8:357-60) mapped a gene for Noonan syndrome to chromosome 12q between D12S84 and D12S366, and demonstrated non-linkage in one family (genetic heterogeneity). Legius et al. (EJHG 1998;6:32-7) narrowed down the interval to a 5-cM region on chromosome 12q24 between markers D12S84 and D12S1341. We report a boy with NS and a cytogenetically balanced translocation of t(2;12)(q37;q24). The clinical diagnosis of NS is definite, but interestingly the 9-yr-old boy displays a partial phenotype without heart defect and without developmental delay. He demonstrates a suggestive face with ptosis (right eyelid only), low-set rotated ears, short neck, and marked webbing of the neck. Additional signs include hirsutism, broad thorax, mild pectus excavatum, small penis, cubiti valgi, height below 3rd centile (-3.5 SD), hearing loss, and retardation of bone age. His intelligence is above-normal with excellent performance in school. We established a lymphoid cell line and computed regional maps with NCBI Entrez Map View. Using FISH mapping and a panel of BAC and YAC clones, we identified BAC clones spanning the breakpoints on chromosomes 2q37 and 12q24, respectively; the breakpoint on 12q24 resides within the 5-cM region between D12S84 and D12S1341. There was no evidence for a chromosomal microdeletion. Our results place the gene for Noonan syndrome (NSI) on chromosome 12q24.2 into or near the breakpoint-spanning BAC clone measuring 187 kb in length. Additional FISH mapping studies followed by positional cloning are in progress.

**Fine Mapping of Autosomal Recessive Progressive Cataract.** *G.D. Billingsley<sup>1</sup>, A.D. Paterson<sup>3</sup>, F.L. Munier<sup>4,5</sup>, A. Balmer<sup>5</sup>, D.F. Schorderet<sup>4</sup>, E. Héon<sup>1,2,3</sup>.* 1) Vision Science Research Program; 2) University of Toronto, Department of Ophthalmology; 3) The Hospital for Sick Children Research Institute; Toronto, Ontario, Canada;; 4) Unit of Oculogenetics and Division of Medical Genetics, CHUV; 5) Hôpital Ophthalmique Jules Gonin, Lausanne, Switzerland.

Cataracts constitute the leading cause of blindness worldwide and the mechanisms of lens opacification remain unclear. The genetics of this condition are complex with 18 loci for autosomal dominant cataract and 2 for autosomal recessive cataract identified to date. Disease causing mutations have been identified in 12 genes. Using a large 3 generation family, we recently mapped a third locus for autosomal recessive cataract to a 14 cM disease interval at 9q13-q22. This unique autosomal recessive cataract is characterized by primarily cortical pulverulent (dust-like) opacities with progressive nuclear and posterior subcapsular involvement and early nuclear sclerosis (browning of the nucleus). Cataract surgery was usually required by the age of 40 years to restore visual function. Further genotype and haplotype analysis has narrowed the disease interval to a 10 cM region defined by D9S1843 and D9S1680 and a potential area of homozygosity that would narrow the interval to 4 cM. This is the second autosomal recessive locus identified but the only one associated with a non-congenital progressive cataract. Also, this new locus does not correspond to a known candidate locus in mice or human. Analysis of the eighteen genes identified in the 10 cM interval suggests that the ARPC gene will be novel. The molecular characterization of this phenotype may shed light on the complex cascade of events modulating lens differentiation and lens opacification in the more common age-related cataracts.

**Narrowing the CLN6 region on chromosome 15.** *H. Gao*<sup>1</sup>, *J. Espinola*<sup>1</sup>, *T. Lerner*<sup>1</sup>, *R-M. Boustany*<sup>2</sup>, *M.E. MacDonald*<sup>1</sup>. 1) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown MA; 2) Pediatric Neurology, Duke University Medical Center, Durham, NC.

Neuronal ceroid lipofuscinoses (NCLs) are an inherited group of fatal neurodegenerative disorders characterized by loss of vision and seizures and the accumulation of an autofluorescent lipopigment in neurons and other cells. The NCLs comprise eight forms classified initially by clinical symptoms and age at onset but now recognized as being due to mutations in distinct genes (CLN1- CLN8). CLN6, the gene for a variant late infantile NCL (vLINCL), has been mapped to chromosome 15q21-23 by homozygosity mapping (Hum Mol Genet 6:591-595, 1997; Neurogenetics 1:217-222, 1998). The genetic region has been progressively narrowed using recombination analyses and homozygosity mapping from 12 cM to 10 cM, between D15S1020 and AFM142VC1 (Mol Genet Metab 66:332-336, 1999), and then to 4 cM between D15S125 and D15S1000 (Mol Genet Metab 66:329-331, 1999). Using multiallele markers, developed from dinucleotide repeat sequences from across the minimal genetic region, we have identified a major haplotype, shared by affected chromosomes from 10 vLINCL families. The coding regions of six candidate genes have been scanned for mutation by single-strand conformational polymorphism (SSCP) analysis. Furthermore, by generating new polymorphic markers from dinucleotide repeats in the genomic DNA sequence we have identified a shared sub-region of the major haplotype that narrows the minimal CLN6 region to less than 1cM. Thus, our results suggest that many vLINCL families may share the same CLN6 defect and, by refining the candidate region significantly focus efforts to identify the CLN6 defect.

**Variations in the CAPN10 gene associate with non-insulin dependent diabetes in Icelanders.** V. Emilsson<sup>1</sup>, S. Halldorsson<sup>1</sup>, G. Thorleifsson<sup>1</sup>, R. Benediktsson<sup>2</sup>, G. Sigurdsson<sup>2</sup>, A. Kong<sup>1</sup>, K. Stefansson<sup>1</sup>, J. Gulcher<sup>1</sup>, V. Gudnason<sup>2</sup>, I. Reynisdottir<sup>1</sup>. 1) deCode Genetics Inc., Lynghals 1, 110-Reykjavik, Iceland; 2) The Icelandic Heart Association, Lagmuli 9, 108-Reykjavik, Iceland.

Approximately 4% of the adult population is affected by non-insulin dependent diabetes (NIDDM), which is characterized by insulin resistance coupled with pancreatic b-cell dysfunction and accompanying chronic hyperglycaemia. The disease is commonly associated with obesity, hypertension and dyslipidemia. Recently, an intronic variation (UCSNP43, G/A) in the calpain 10 gene (CAPN10 maps within the NIDDM1 locus at 2q), encoding a cysteine protease, was found to be significantly associated with NIDDM susceptibility among Mexican Americans. Moreover, the at-risk genotype (G/G) has been associated with decreased rates of insulin-stimulated glucose disposal as well as reduced CAPN10 mRNA expression in skeletal muscle. We have analysed association of variations in CAPN10 with high fasting plasma glucose (FG<sup>37</sup>mM) in Icelandic subjects. The study groups consist of 285 NIDDM patients (FG<sup>37</sup> mM; age (yr)=71±11; BMI=27±4) and a random sample of 267 unrelated and undiagnosed subjects. Frequencies of five intronic variations in the CAPN10, including the causal variation UCSNP43, were genotyped by the fluorescence polarization method or gel electrophoresis. A major allele frequency of the different variations in the random sample was between 64 and 90%. The association with the variation in UCSNP43 is statistically significant (two-sided p-value=0.027), whilst the association with a 32 bp repeat variation (UCSNP19) and an A/G variation (UCSNP62) was marginally significant (p=0.056 and p=0.055, respectively). Frequencies of the associated at-risk allele UCSNP43, in both study groups, were similar to that found among Mexican Americans. Moreover, the at-risk allele conferred an estimated population-attributable risk that accounts for 26% of NIDDM cases in the study population. Carriers of one or both at-risk alleles had an increased risk, compared to non-carriers, or 11 and 61%, respectively. Thus, by independent observations, our study confirms that variations in CAPN10 influence susceptibility to NIDDM.

**Searching for diabetic nephropathy susceptibility genes in the Finnish population using linkage and association studies.** *B. He<sup>1</sup>, A-M. Österholm<sup>1</sup>, J. Pitkäniemi<sup>2</sup>, L. Liu<sup>1</sup>, T. Berg<sup>1</sup>, J. Tuomilehto<sup>2</sup>, K. Tryggvason<sup>1</sup>.* 1) Division of Matrix Biology, MBB, Karolinska Institute, Stockholm, Sweden; 2) The Finnish National Public Health Institute, Helsinki, Finland.

Microvascular changes (microangiopathy) are one of the main complications of diabetes and they are considered to be the primary cause of kidney and eye damages that affect diabetic patients. Diabetic nephropathy (DN) is characterized by thickening of the glomerular basement membrane, and clinically it is manifested by persistent proteinuria and end stage renal disease. DN affects 35% of patients with insulin-dependent diabetes mellitus (IDDM). Family studies have provided evidence for genetic determinants of nephropathy. So far, candidate gene studies have not been successful and no genes have been reported to be directly associated with DN. A locus linked to DN on chromosome 3q has been reported in an American Caucasian population. This finding was confirmed by our ongoing genome wide scan. In follow-up linkage studies, we genotyped 15 markers spanning 64 cM on chr3q in 50 Finnish DSPs. A MLS of 3.0 was obtained by multipoint linkage analysis. Subsequently, 1-lod-unit support interval of 9 cM was determined. A physical map (about 9.7 Mb) of the critical region was constructed based on the Celera database. In total, the database revealed approximately 140 genes, both identified and predicted. Twenty-four multi-allelic markers in this region were localized, and used for further population-based association studies. In a dataset of 184 Finnish cases and 83 controls, two adjacent markers showed statistically significant association ( $p=4.6 \times 10^{-7}$  and 0.01). We identified and localized 5 new repeats within these two adjacent markers by sequence searching. Two of them also showed significant association. Therefore, the critical region most likely bearing the gene might be 300 Kb in length. By DNA analysis, a known gene and several other predicted genes were found in the region. The known gene is a very promising candidate, since expression was detectable in kidney. An intronic SNP of the known gene gave a strong association ( $p=0.0003$ , OR=2.0). Association studies in a Danish population are ongoing.

**Analysis of chromosome 1 for SLE susceptibility loci in a new set of 69 multicas families.** *B. Johanneson<sup>1</sup>, G. Lima<sup>2</sup>, M. Alarcon-Riquelme<sup>1</sup>, Collaboration on the genetics of SLE<sup>1</sup>, BIOMED II collaboration on the genetics of SLE and Sjogrens Syndrome<sup>1</sup>.* 1) Dept. of Genetics and Pathology, Uppsala University, 751 85 Uppsala, Sweden; 2) Dept. of Immunology/Rheumatol., Inst. Nacl. Cien. Med. Nutr., 1400 Mexico DF, Mexico.

**AIM** SLE is a complex and heterogeneous disease with a genetic component. Combinations of susceptibility genes are likely to exist in different populations. Several susceptibility loci have been identified in different genomescans, with varying results. Our aim was to confirm susceptibility regions on chr 1, using a new set of 69 multicas families (422 individuals ) from defined populations, and determine if ethnical differences lie behind the variable results.

**RESULTS** A total of 5 regions with markers showing suggestive linkage ( $LOD > 2,2$ ) were detected using parametric two-point linkage analysis on 41 microsatellites for chr. 1. All regions have been reported previously. Two show contributions from all populations (1q23-24 and 1q25-31). 1p36 and 1p21-22 were contributed mainly from South European and American families (mainly from Mexico and Colombia). The 1q41-44 region has contributions from mainly European and Chinese families. The study shows that by using clear defined populations, it is possible to determine the genetic effect contributed by each population to each susceptibility region.

Members of the collaboration on the genetics of SLE: Mauro Galeazzi, Sergio Migliaresi, Domenico Sebastiani, Ornella de Pit, Antonio Iglesias, Kok-Yok Fong, Ralph Williams jr, K. Boki, Maria Kastorida Ignacio Garcia, and Donato Alarcn-Segovia.

Members of the BIOMED II collaboration: Caroline Gordon, Helga Kristjansdottir, Anne-Isine Bolstad, Elisabet Svenungsson, Iva Gunnarsson, Kristjn Steinsson, Gunnar Sturfelt, Lennart Truedsson and Roland Jonsson.



**Association studies involving multiple variant alleles of the low affinity Fcγ Receptors.** *V. Magnusson<sup>1</sup>, J. Odeberg<sup>2</sup>, M.E. Alarcón-Riquelme<sup>1</sup>*. 1) Dept. of Genetics and Pathology, Rudbeck laboratory, Uppsala University, Sweden; 2) Dept. of Biochemistry and Biotechnology, Royal Institute of Technology, Sweden.

The aim with our study is to determine the distribution of FcγRs alleles in ethnically diverse populations and investigate their influence on susceptibility for developing autoimmune disorders, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). RA and SLE are complex systemic disorders whose expression is influenced by multiple genes and environmental factors. Identification of genetic risk factors underlying specific disease manifestations, for example lupus nephritis would be of great importance in order to dissect the genetic component in these diseases. We have focused on the low-affinity receptors for IgG because of their importance in the immune response and regulation. Variant genotypes of these receptors have been reported to differ between disease and control populations. Several single nucleotide polymorphisms (SNPs) at the FcγRIIA, FcγRIIA and FcγRIIB loci were analysed, including SNPs of functional or clinical importance as well as new SNPs identified by in silico search and sequencing. The study populations consist of 300 Mexican and Swedish RA patients, 300 Mexican and Swedish SLE patients, and 400 ethnically matched controls, for each group. Genotyping was done by allele-specific PCR, restriction enzyme digestion or pyrosequencing. P-values and odds ratios (OD) with 95% confidence interval were estimated using the SAS procedure FREQ. We have found an association in the Swedish as well as in the Mexican population between the R-allele at the FcγRIIA-131 locus and SLE, ( $p=0.01$  and  $p=0.001$ , respectively) in female patients. This association was not found in the patients with RA.

**Family based association tests for censored data with pedigrees.** *O. Mokliatchouk.* Mathematics & Computer Science, Drexel University, Philadelphia, PA.

Family based association tests are currently developed for categorical, quantitative and censored traits with nuclear families and for categorical and quantitative traits with pedigrees. Although these methods can be applied to data on traits with variable age at onset and pedigrees, they are not efficient, since they do not incorporate all available information. The proposed tests are extensions of a family based association test to data on traits with variable age at onset and pedigrees. Proportional hazards regression models are used to motivate the methods. Conditioning on the minimal sufficient statistics for association in the absence of linkage is used to avoid confounding due to factors other than linkage. The tests involve estimating survival function of the censored trait given the observed marker genotypes and unobserved trait genotypes. A theory for such estimation is developed. Results of simulations that explore the performance of these procedures are described. The procedures are illustrated through an analysis of data on APOE genotype, a mutation of alpha-2 macroglobulin gene (A2M) and Alzheimers disease.

**Candidate genes for late-onset Alzheimers disease on chromosome 12.** *E. Luedeking-Zimmer*<sup>1</sup>, *S.T. DeKosky*<sup>1,2</sup>, *M.I. Kamboh*<sup>1,2</sup>. 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Psychiatry, University of Pittsburgh, Pittsburgh, PA.

Alzheimers disease (AD) is a genetically heterogeneous neurodegenerative disorder. To date, apolipoprotein E (apoE) is the only universally accepted susceptibility gene for late-onset AD. ApoE accounts for less than 50% of the risk of late-onset AD, indicating the likelihood of other unknown susceptibility loci. Recently, linkage studies have provided strong evidence for a late-onset locus located on chromosome 12 among non-APOE\*4 carriers. A broad 50 cM pericentromeric region between markers D12S358 (12p13) and D12S1632 (12q13) is the predicted region of a putative gene(s). In this study, we have examined 9 polymorphisms in 5 candidate genes located in this region in a large case-control cohort (597 cases and 530 controls). The examined candidate genes include, -2 macroglobulin (A2M), complement component 1R (C1R), and oxidized-LDL receptor (OLR1) in the 12p13 region, and LDL receptor-related protein (LRP1), and transcription factor LBP-1c/CP2/LSF in the 12q13 region. No association was observed with the 2, 3, and 1 polymorphisms examined in the A2M, LRP1, and C1R genes, respectively. A modest association was observed with a 3 UTR polymorphism in the transcription factor LBP gene with and odds ratio (OR) of 0.65 (95% CI 0.45-0.96;  $p=0.03$ ). All exons and intron-exon boundaries of the OLR1 gene were screened for mutation detection by D-HPLC and DNA sequencing and we identified 3 novel polymorphisms in intron 4, intron 5 and in the 3UTR. All three polymorphisms were in linkage disequilibrium and showed a significant association with AD among non-APOE\*4 carriers, with the strongest effect observed for the 3 UTR polymorphism (OR=0.55, 95% CI 0.36-0.83;  $p=0.005$ ). Our data suggest the existence of two putative genes for AD on chromosome 12p and 12q regions.

**A Genome-Wide Scan of QTLs for Obesity-Related Phenotypes Using Sibling Pairs.** *T. Niu*<sup>1,2</sup>, *Z. Fang*<sup>3</sup>, *L. Duprat*<sup>4</sup>, *X. Xu*<sup>1</sup>, *D. Chen*<sup>5</sup>, *J. Jin*<sup>2</sup>, *S. Lewitzky*<sup>4</sup>, *J. Meyer*<sup>4</sup>, *X. Xu*<sup>1,2,6</sup>. 1) Prog Population Genetics, Harvard Sch Public Health, Boston, MA; 2) Institute of Biomedicine, Anhui Medical University, Hefei, Anhui, China; 3) Anqing Public Health Bureau, Anqing, Anhui, China; 4) Genetics Division, Millennium Pharmaceuticals, Inc., Cambridge, MA; 5) Anhui Meizhong Institute for Biomedicine and Environmental Health, Anqing, Anhui, China; 6) Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

To identify quantitative trait loci (QTLs) that contribute to obesity, we performed a genome-wide scan of obesity-related phenotypes using data collected from a total of 154 nuclear families (407 siblings) in Anqing, China, resulting in 416 concordant or discordant sibpairs for body mass index (BMI) (i.e., the siblings are either at the lowest or the highest decile of the age- and gender-specific BMI distributions in a general population of the same community). A total of 502 microsatellite markers were genotyped in 22 autosomes. Using the XWXW computer program that implements the unified Haseman-Elston algorithm, we detected several moderate linkage signals for covariate-adjusted BMI on chromosomes 6p ( $Z = 2.84$ ,  $p=0.002$ ), 7q ( $Z=2.82$ ,  $p=0.002$ ), and 17q ( $Z=2.72$ ,  $p=0.003$ ). With respect to covariate-adjusted waist circumference, a peak was observed on chromosome 2p ( $Z=2.82$ ,  $p=0.002$ ). Regarding covariate-adjusted waist-to-hip ratio, a suggestive signal was found on chromosome 14q ( $Z=2.61$ ,  $p<0.005$ ). Follow-up studies using denser markers are needed to pursue those linkage signals located near known biological candidate genes for obesity, and those that overlap with positive linkages reported previously.

***IGF2R as a candidate gene for autoimmune diabetes.*** *J.A. McCann, Y. Xu, L. Marchand, C. Polychronakos.*  
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Genetic susceptibility to Type I diabetes is a polygenic trait. The HLA locus on 6p21 (IDDM1) and the insulin locus on 11p15.5 (IDDM2) together account for a little more than half the genetic effect. The remaining genetic susceptibility is determined by several other loci with smaller effects, some of which have been localized by sib-pair linkage analysis. Among these is IDDM8 on 6q26. Identification of the gene involved in IDDM8 requires systematic scanning of the region encompassed. We selected IGF2R, encoding the IGF-II receptor, because of the importance of the IGF system in both beta cell regeneration and functioning of the immune system. Using eight polymorphisms found throughout the IGF2R, we studied a group of diabetic children from the Montreal area, including those of French Canadian background, a homogenous population in which the small number of ancestral chromosomes maximizes the probability of finding linkage disequilibrium between markers and disease. Deviation of allele transmission from heterozygous parents to diabetic children from the expected 50% was examined. Since IGF2R is imprinted in the mouse, and under some circumstances in the human, we examined paternal and maternal transmissions separately. Of the polymorphisms studied, only one in exon 16 showed a transmission disequilibrium between mothers and fathers to their diabetic children. This effect was most strongly seen in the French Canadian population. Of the 23 heterozygous French Canadian mothers, 17 transmitted the G allele and only 6 transmitted the A allele ( $p=0.017$ ). In fathers, the corresponding numbers are 10 and 10 (NS). When non-French Canadian populations were examined, the same results were found in families of Italian descent but not in an internationally mixed sample set. Our data suggest that IGF2R, or a near-by imprinted gene in linkage disequilibrium with our marker, may be the gene involved in IDDM8. If it is IGF2R itself, it must be universally imprinted in some tissue and/or developmental stage crucial to autoimmune diabetes.

**Candidate gene polymorphisms and type 2 diabetes among Samoans: significant associations at HNF-1b and GCK loci.** *D.T. Smelser<sup>1</sup>, X. He<sup>1</sup>, S.T. McGarvey<sup>2</sup>, D.E. Weeks<sup>3</sup>, R. Indugula<sup>1</sup>, R. Deka<sup>1</sup>.* 1) Environmental Health, University of Cincinnati, Cincinnati, OH; 2) International Health Institute, Brown University, Providence, RI; 3) Human Genetics, University of Pittsburgh, Pittsburgh, PA.

The contemporary adult Samoan population of Polynesia has a very high prevalence of type 2 diabetes, ranging from 3 and 5% among men and women, respectively, in the independent country of Samoa to 25 and 15% among men and women, respectively, in American Samoa. We have tested for association between type 2 diabetes and eight candidate gene polymorphisms (PC1, PPAR $\gamma$ 2, FABP2, HNF-1b, GCK, NPY, ADR $\beta$ 3, UCP3) in a sample of 96 controls without type 2 diabetes and 180 affected individuals. The controls were derived from both polities, which showed no allele frequency differences; the affected individuals were drawn from American Samoa. Genotype frequencies were found to be in conformity with Hardy-Weinberg (H-W) expectations excepting the affected samples at the FABP2 locus. We performed a chi-square based association test and Fisher's Exact Test using genotype frequencies, which showed significant difference between cases and controls at two loci, HNF-1b ( $p = 0.027$ ) and GCK ( $p = 0.009$ , two-tailed). Both of these genes are implicated in the pathway of glucose metabolism (Bach et al. 1991. *Nucleic Acids Res* 19:3553; Matschinsky 1990. 39:647). Interestingly, the affected samples at the FABP2 locus show significant deviation from H-W proportions, whereas the controls are at the expected equilibrium. This is indicative that the FABP2 locus may be associated with type 2 diabetes among the Samoans. We are testing other candidate loci as well as looking at other polymorphisms within each locus. Supported by NIH grants AG09375, HL52611, DK55406, and DK59642.

**Statistical Analysis of Direct Identity-by-descent Mapping.** *B. Yakir*<sup>1</sup>, *D. Siegmund*<sup>2</sup>. 1) Dept Statistics, Hebrew Univ, Jerusalem, Israel; 2) Dept. of Statistics, Stanford Univ, Stanford, CA.

Genomic mismatch scanning (Nelson et al., 1993) allows one to determine directly, without genotyping, the regions inherited identical-by-descent (IBD) by pairs of relatives, including pairs of distant relatives of unknown relationship. Application of this technique to ostensibly unrelated, but putatively distantly related, pairs of affecteds has been suggested by Cheung et al. (1998) as a method for fine genetic mapping by identification of regions shared IBD by a larger number of affected pairs than would be expected by chance. Several different methods for statistical analysis of such data were discussed by Grant et al. (1999) under restrictive assumptions about the form of the data. In this paper we (i) model the alternating genomic regions of IBD and non-IBD of a relative pair as a continuous time two state Markov chain having intensities determined by the relationship (which in general is unknown), (ii) derive the score statistic to test for linkage, (iii) describe a method to approximate chromosome wide or genome wide p-values, and (iv) obtain confidence regions for map location. To avoid problems associated with lack of knowledge of the relationship of the affected pairs and hence the intensities in (i), we develop our p-value approximation and confidence regions by conditioning on an appropriate sufficient statistic for each pair, namely the IBD status at an initial position on a chromosome, the proportion of the chromosome shared IBD, and the number of transitions between regions of sharing and non-sharing. This allows us in effect to estimate important parameters of the average relationship in our sample from the observed pattern of IBD sharing without directly estimating the relationships themselves.

**Linkage disequilibrium (LD) domain mapping identifies PSORS1 candidate regions for psoriasis susceptibility.**

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Psoriasis is a complex inflammatory skin disease affecting on average 2% of all populations, it is characterised by keratinocyte hyperproliferation, immune cell infiltration and angiogenesis. Previous genome wide scans have identified a number of putative susceptibility loci, with linkage to a region at the boundary of the MHC class III/I at 6p21 representing the major and consistent finding. LD mapping has refined this to an approximately 300kb region around HLA-C. To further characterise this region we have sequenced a total of 64kb between and including HLA-C and the CDSN in 8 psoriatics to identify SNPs that may be of biological relevance to psoriasis. SNPs that were identified with a minor allele frequency greater than 0.2 within the coding region of the characterised genes OTF3, SC1, HCR and CDSN were then genotyped in a UK cohort of caucasian affected offspring trios (n=175) and family based tests of association were performed. Strong allelic association was seen for SNPs within OTF3 ( $p=4.3 \times 10^{-6}$ ) HCR ( $p=8.7 \times 10^{-7}$ ) and CDSN ( $p=4.9 \times 10^{-6}$ ). Specifically association arises through SNPs leading to non-conservative amino acid substitutions with exception of OTF3 in which the associated SNP is silent. Analysis of LD between SNPs in the unrelated parents showed strong LD between OTF3 and HCR ( $D'=0.997$ ) explaining the association with the silent SNP in OTF3, however both these genes show a lack of LD with CDSN SNP 1243 ( $D'=0.015$ ) in this cohort indicating that association at CDSN is independent of HCR/OTF3. Association of these non-conservative changes at the protein level identify both HCR and CDSN as potential functional candidates. A further 100 SNPs have been identified in this region enabling definition of the extent of linkage disequilibrium in this region and elucidate the relationship between these genes and polymorphisms to psoriasis.



**LINKAGE AND ASSOCIATION OF A FUNCTIONAL SINGLE NUCLEOTIDE POLYMORPHISM (SNP) IN THE FcgRIIA GENE WITH SLE.** *J.C. Edberg<sup>1</sup>, C.D. Langefeld<sup>2</sup>, K.L. Moser<sup>3</sup>, J. Kelly<sup>4</sup>, J.M. Kaufman<sup>4,5</sup>, S.S. Rich<sup>2</sup>, J.B. Harley<sup>4,5</sup>, R.P. Kimberly<sup>1</sup>, the SCOR in Genetics in SLE<sup>1,2,3,4,5</sup>.* 1) Univ of Alabama at Birmingham, AL; 2) Wake Forest University, Winston-Salem, NC; 3) Univ Minnesota, Minneapolis, MN; 4) Oklahoma Med Res Foundation, Oklahoma City, OK; 5) Univ Oklahoma Health Sci Ctr & US Dept Veterans Affairs, Oklahoma City, OK.

Genome scans of multiplex SLE families have identified multiple loci linked to SLE. Two candidate genes in the 1q23 region, FcgRIIA and FcgRIIA, contain SNPs in the coding region that alter the binding of IgG. Case-control studies provide evidence of an assoc between these SNPs and SLE. To test for family-based assoc, we genotyped and analyzed 126 multiplex families with SLE (61% Cauc, 32% Afr-Amer); genotyping was done independently at 2 sites. Multipoint non-parametric linkage analysis of these SNPs provides evidence for linkage at both loci (FcgRIIA:NPL score=2.54  $p < 0.006$ ; FcgRIIA: NPL score=2.62,  $p < 0.005$ ). Analysis of family-based assoc using the TDT and the PDT revealed strong assoc at the FcgRIIA locus with little or no evidence of assoc at the FcgRIIA locus. These two SNPs are in significant linkage disequilibrium ( $p < 0.0009$ ). There was no evidence of an assoc with an extended haplotype ( $p = 0.06$ ). Based on these results, the association between these SNPs and SLE is primarily due to the FcgRIIA SNP where the risk allele was transmitted 61 times and not transmitted 28 times (TDT-based O.R.=2.17,  $p = 0.005$ ). This study implicates FcgRIIA in susceptibility to SLE.

	Caucasians	(n=77)	Afr-Amer	(n=40)	All	(n=126)
	TDT	PDT	TDT	PDT	TDT	PDT
FcgRIIA	p=0.388	p=0.097	p=0.074	p=0.437	p=0.134	p=0.089
FcgRIIA	p=0.012	p=0.044	p=0.016	p=0.020	p=0.0005	p=0.002

**Evaluation of expression and genetic variations of Th1-type cytokines in peripheral blood mononuclear cells from asthmatic patients in Iceland.** *I.F. Birkisson<sup>1</sup>, U.S. Bjornsdottir<sup>1</sup>, E. Halapi<sup>2</sup>, D.L. Shkolny<sup>2</sup>, T. Arnason<sup>2</sup>, D. Gislason<sup>1</sup>, T. Gislason<sup>1</sup>, H. Hakonarson<sup>1,2</sup>.* 1) Medical Department, University of Iceland, Reykjavik, Iceland; 2) deCODE Genetics Inc., Reykjavik, Iceland.

Recent evidence support the concept that deficiency in the Th1 cytokine pathway is associated with increased susceptibility to allergic airways disease such as asthma. This study examined whether i) single nucleotide polymorphisms (SNPs) exist in the promoter region of the two subunits forming the IL-12 gene in asthmatic patients, ii) mRNA and protein expressions of STAT4, IL-12, IFN-g, and their receptors are altered in asthma, and; 3) genetic linkage to the IL12 gene is present in asthmatic families in Iceland. The promoter region of the IL12 gene was sequenced in 94 asthmatic patients and 94 non-asthmatic controls. Linkage was examined in 140 families that included over 400 asthmatic patients and 400 of their unaffected relatives. We sequenced 3.5 kb of genomic DNA and examined for induced changes in mRNA and protein expressions of isolated PBM cells in 15 asthmatic patients and 15 non-asthmatic controls, using kinetic PCR, ELISA and flow cytometry. The patients phenotypes were determined by 3 Allergists in accordance with the ATS criteria. Age ranged from 18-64 years. Spirometry, IgE levels, methacholine challenge test and skin test was performed in all patients. The results demonstrate, no evidence of alteration of the Th1 pathway in our asthmatic patients. No polymorphism was detected in the two promoter regions of the IL-12 gene and no linkage was detected to microsatellite markers in close association of the two IL-12 genes, on chromosomes 3 and 5 respectively, in the asthmatic patients. Moreover, we found no differences, in neither mRNA or protein expressions in the IL-12 pathway, between patients and controls, using the experimental techniques described above. Taken together, we found no evidence of altered Th1 type cytokine expression or action in our asthmatic patients, and we uncovered no genetic differences or linkage to the IL-12 gene. We conclude that this study fails to support the concept that decrease in Th1 type cytokine response is a primary event in asthma.

**Comparison of Haseman-Elston Methods for Assessing Parent-of-Origin Effects in Linkage Analysis.** *R.L.*

*Hanson, S. Kobes, R.S. Lindsay, W.C. Knowler. DAES, NIDDK, Phoenix, AZ.*

We recently developed a method to assess parent-of-origin effects in linkage analysis of quantitative traits in sibships. This method can use variance components (VC) or Haseman-Elston (HE) methods, but the VC method is more powerful than the classic HE. As recently proposed modifications of the HE method enhance its power for nonimprinted loci, we analyzed simulated data to assess the properties of these modified methods for assessment of parent-of-origin effects.

Data were simulated for a variety of models for 956 siblings in 263 nuclear families who had participated in a linkage study. The HE linkage method involves regression analysis of a function (F) of the trait values in the sibling pair against the proportion of marker alleles shared identical by descent, partitioned into maternally- and paternally-derived components. In the classic HE, F is the squared trait difference (HE-SD). We evaluated proposed modifications in which F is the trait product corrected for the grand mean (HE-PGM), the product corrected for the family-specific mean (HE-PFM) or a weighted combination of the squared difference and squared sum (HE-COM).

Power of the HE-PFM and HE-COM methods was comparable to the VC method, while HE-SD and HE-PGM were somewhat less powerful. For example, in 500 replicates of a maternally-expressed locus (heritability=30%), LOD > 3 with the maternal chromosome was observed in 84% of VC, 72% of HE-SD, 75% of HE-PGM, 86% of HE-PFM and 84% of HE-COM analyses, and none of the methods had LOD > 3 to the paternal chromosome in any replicate. However, in data generated for a nonimprinted locus, type I error rates for a test of equal parental effects were inflated for all HE methods: a nominally significant result ( $p < 0.05$ ) was observed for 10% of HE-SD, 11% of HE-PGM, 14% of HE-PFM and 12% of HE-COM analyses, compared with 4% of VC analyses.

These analyses suggest that these modifications of the HE method do not appear to be superior to the VC method for assessing parent-of-origin effects.

**Nonparametric disequilibrium mapping of functional sites using haplotypes of multiple tightly linked single-nucleotide polymorphism (SNP) markers.** *R. Cheng<sup>1</sup>, J.Z. Ma<sup>2</sup>, F.A. Wright<sup>3</sup>, S. Lin<sup>4</sup>, X. Gao<sup>3</sup>, D. Wang<sup>3</sup>, R.C. Elston<sup>5</sup>, M.D. Li<sup>1</sup>.* 1) Department of Pharmacology; 2) Department of Preventive Medicine, University of Tennessee, Memphis, TN; 3) Division of Human Cancer Genetics; 4) Department of Statistics, Ohio State University, Columbus, OH; 5) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH.

When a gene has been implicated in causing disease, it is desirable to exhaustively survey allelic variation within it for any association to disease. As the genotyping speed and efficiency of single nucleotide polymorphisms (SNPs) increase, such studies will fuel increasingly comprehensive tests of the hypothesis that common variants contribute significantly to the risk of common diseases. Using the SNP map, it becomes possible to evaluate the extent to which common haplotypes contribute to the risk of disease. In this study we propose a procedure for mapping functional sites or regions within the sequence data of candidate genes using multiple SNPs. Based on a case-parent trio family design, we use expectation maximization (EM) algorithm-derived haplotype frequency estimates of multiple tightly linked SNPs from both unambiguous and ambiguous families to construct a contingency statistic *S* for linkage disequilibrium analysis. In our procedure, window searching for functional sites or regions can cover any number of loci without limitation except for that of computer storage. Within a window, all possible widths of haplotypes are utilized to find the maximum statistic *S* for each site (or locus). Furthermore, this method can be applied to regional or genome-wide scanning in order to study linkage disequilibrium using SNPs. The proposed procedure was applied to scan simulated sequence data and we found that the functional sites for candidate genes could be successfully identified.

**Lack of association between Common ATM polymorphisms and breast cancer risk.** *F. Durocher*<sup>1,2</sup>, *L. Williams*<sup>2</sup>, *S. McBride*<sup>2</sup>, *J. Hall*<sup>3</sup>, *S. Angele*<sup>3</sup>, *B. Ponder*<sup>2</sup>, *A. Dunning*<sup>2</sup>, *D. Easton*<sup>1</sup>. 1) CRC Genetic Epidemiology Unit, and; 2) Department of Oncology, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge, UK, CB1 8RN; 3) International Agency for Research on Cancer, 150 Cours Albert Thomas, 69008, Lyon, France.

Ataxia-telangiectasia (AT) is a recessive syndrome characterized by cerebellar degeneration, immunological defects and cancer predisposition. Most cases of the disease are the result of mutations in the ATM gene. Epidemiological studies have shown an increased risk of breast cancer in obligate heterozygotes in AT families. In the present study, we investigated the potential association of sporadic breast cancers with three common ATM polymorphisms: P1054R in exon 23, D1853N in exon 37 and IVS\_48 in intron 48.

Genotypes in these three polymorphisms were determined in a series of up to 2592 sporadic breast cancer cases and 1964 controls subjects, from the East Anglian region, UK, by a fluorescent method. The associations between specific genotypes and the risk of developing breast cancer were performed by logistic regression analysis to calculate odds ratio (OR) and floating confidence intervals (FCI).

No significant association with breast cancer was found with any of the three polymorphisms analysed individually; (ORs: 1.25, 95% CI 0.82-1.75; 0.98 95% CI 0.85-1.13 and 0.97 95% CI 0.85-1.12) for heterozygotes of the P1054R, D1853N, and IVS\_48 polymorphisms, respectively. Furthermore, no particular haplotype or combinations of genotypes appeared to be associated with a significantly increased risk. However, the D1853N polymorphism exhibited significant departure from Hardy-Weinberg equilibrium for both the breast cancer cases and the controls ( $p=0.0015$  for cases and controls combined), suggestive of a selective advantage in heterozygotes.

**Complex and Unstable Haplotypes of the *CDKN1A* Gene.** *L. Geller*<sup>1</sup>, *G.P. Larson*<sup>1</sup>, *C. Ouyang*<sup>1</sup>, *S. Flanagan*<sup>2</sup>, *T.G. Krontiris*<sup>1</sup>. 1) Division of Molecular Medicine, Beckman Research-City of Hope, Duarte, CA; 2) Division of Neuroscience, Beckman Research-City of Hope, Duarte, CA.

As a major downstream target of TP53, CDKN1A (p21, *CIP1*, *WAF1*) inhibits the G1-to-S cell cycle checkpoint and serves as a potent negative regulator of the mammalian cell cycle. In a recent analysis of breast cancer risk, we identified four rare, potentially pathogenic SNPs within the promoter, coding, and 3' untranslated regions of *CDKN1A*. In addition to risk-associated sequence variants, other SNPs were quite prevalent within the promoter and flanking regions. Through sequencing of cloned PCR fragments, we identified 20 complex haplotypes from breast cancer patients and unaffected controls. The four potentially cancer-associated SNPs were present on 3 different haplotypes. Approximately 80 SNPs, insertions, deletions, and microsatellites were present within the 28 kb region characterized. We observed at least ten independent crossovers and many gene conversion events in the region within and surrounding *CDKN1A*. Linkage disequilibrium extended over the entire 28 kb region, and included two microsatellites. These results--the complex haplotype architecture at this locus, as well as the distribution of rare, cancer-associated variants over multiple haplotypes--have important implications for genome-wide association tests based on linkage disequilibrium of SNPs.

**Linkage and linkage disequilibrium fine-scale mapping of 16 cM region on chromosome 4p16-15.2 in pedigrees multiplex for human systemic lupus erythematosus.** *C.L. Gray-McGuire<sup>1,2</sup>, J. Kelly<sup>1</sup>, H. Tiwari<sup>2</sup>, J.B. Harley<sup>1</sup>*. 1) Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Case Western Reserve University, Cleveland, OH.

Systemic lupus erythematosus (SLE) is a complex autoimmune disorder involving at least hormonal, environmental, and genetic factors. To date, six genome scans of linkage to SLE have been conducted and more than fifteen regions of interest identified. However, only three of these regions have both attained a level of significance equivalent to a lod score of 3.3 and been confirmed in an independent study, one of which is 4p16-15.2. This linkage effect was first identified in a collection of 77 European American pedigrees multiplex for SLE and then confirmed in an independent collection of 148 European American affected sibling pairs. Subsequent to this confirmation, genotypes were collected for eleven microsatellite markers spanning a 16 cM region (average of 1.1 cM spacing) on 4q16-15.2. Haplotypes were scanned for double recombinations and the genotypes of potentially erroneous individuals were reexamined and either verified or removed. Fine scale multipoint model-free linkage analysis of 270 European American sibling pairs (affected and unaffected) and 101 European American affected relative pairs resulted in a p-value less than 0.01 and lod score greater than 4, respectively. Linkage disequilibrium analysis also indicates an effect in this region with significant results over a 3.1 cM region on 4p16. These results not only confirm linkage in this region, but suggest that the original effect identified to 4p16-15.2 can be isolated to 4p16, the p-terminal end of chromosome 4.

**Using Run Test to Assess Global Significance of Association between a Disease Gene and Multiple SNPs.** C.S.J. Fann<sup>1</sup>, I.B. Lien<sup>2</sup>, W.C. Chou<sup>2</sup>, C.J. Chang<sup>3</sup>. 1) Epidemiology & Public Health, Inst Biomed Sci, Acad Sinica, Taipei, Taiwan; 2) Department of Mathematics, National Chang Hua University of Education, Taiwan; 3) Department of Medical Research, National Taiwan University Hospital, Taiwan.

Association tests (family based or not) between a putative disease gene and a large number of single nucleotide polymorphism (SNP) have gained tremendous popularity recently. With many SNPs, if one examines association using one marker/haplotype after another along the genetic map, will produce large number of testing results and inflate type I error. Using Bonferroni's procedure to adjust problems arisen from multiple comparisons is too conservative since tests at nearby marker loci may be dependent. In the presence of linkage disequilibrium, positive results of association should appear correlatively between a disease gene and flanking SNPs. In this study, we propose to use "longest run" statistics in "run test" to assess the global association between multiple SNPs and a putative disease gene. If p-value obtained from an association test is smaller than a preset threshold (e.g.  $p < 0.05$ ) then assign an index of 1, otherwise assign 0. In general, three situations are observed: a) many 0 and a few 1, b) 1 and 0 appear randomly, c) many 1 and a few 0. Data from a string of such indices are tested for randomness first to exclude situation b. If non-randomness is found and with more index of 1 (situation c) then the probability observing a longest run of index of 1 is used to assess global association. In reality, the indices in the disease gene susceptible region, although likely to be ones, may contain a small number of zeros due to the chosen threshold. The conventional run test based on perfect longest run may be too conservative. Therefore, a new class of run, which refers as *k-zero* run with fixed length of  $n$ , denoted by  $L_{k,n}$  is developed. Furthermore, let  $\max L_{k,n}$  denotes the longest *k-zero* run for the whole sequence then a segment of a chromosome is considered susceptible if  $\max L_{k,n} > C$ , where  $C$  satisfies  $P(\max L_{k,n} > C | H_0) < \alpha$  and  $H_0$  is the null hypothesis of randomness. Both the exact distribution and asymptotic properties of this test is investigated.



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**A regression-based haplotype TDT for quantitative traits in pedigree data.** *V. George*<sup>1</sup>, *C. Chen*<sup>2</sup>, *H. Tiwari*<sup>2</sup>. 1) Dept of Biostatistics, Medical College of Wisconsin, Milwaukee, WI; 2) Case Western Reserve University, Cleveland, OH.

Recently, the transmission/disequilibrium tests (TDT) using single marker have been generalized in various ways to allow for haplotypes made of several tightly linked markers. Analysis of linkage using transmission of haplotype is more informative than that using single marker, and therefore should provide better statistical power. In this presentation, we propose a haplotype-based TDT for quantitative traits using a multiple regression approach. The proposed method allows the trait to be correlated, and also it allows adjustment for relevant covariates in the model. We investigate the statistical power and validity of the method by simulating haplotypes at various distances from the quantitative trait locus.

**SNP Density Requirements in Mapping Genes for Complex Diseases.** *Y-S. Chen, N. Akula, T.G. Schulze, S. Potluri, F.J. McMahon.* Dept Psychiatry, Univ Chicago, Chicago, IL.

The required marker density to detect association with disease has been the subject of considerable debate. Since the extent of linkage disequilibrium (LD) in outbred samples has been estimated by different studies to range from about 3 kb to greater than 100kb, the number of SNPs needed to screen a 10 MB target region may vary from 50 to 1700. To decide the appropriate SNP density for further study of a bipolar disorder linkage region on chromosome 18q21-23, we estimated LD between SNP markers on the first finished long-range sequenced in the region: AC006203. Public databases yielded 70 SNPs; 7 additional SNPs were discovered in-house by resequencing 8-20 unrelated Europeans. These 77 SNPs were then screened in a panel of 20 unrelated European CEPH founders using single-base extension methods. 37% (26 of 70) of the public SNPs and 100% (7 of 7) of the in-house SNPs yielded at least one heterozygote. The 33 polymorphic SNPs all mapped back uniquely to the original clone at a mean density of about one SNP per 5.6 kb (range 239 -11,082 bp). We selected the 33 SNPs with at least one detectable heterozygote for genotyping in an additional set of 50 unrelated European CEPH founders. Overall, genotype data was 99.6% complete. Minor allele frequencies averaged 0.25 (range 0.01- 0.50). Haplotype frequencies, p-values, and LD parameters for all possible pairwise comparisons were estimated with EH+ (Zhao et al 2000). LD decayed over physical distance in a reasonably predictable manner, falling from 1.0 to 0.5 over about 62 kb. These data agree closely with those of Reich et al. 2001, which suggests that the 18q region is not atypical. Statistically-significant LD ( $p < 0.05$ ) was detectable between 80% of marker pairs 2 kb apart and 60% of marker pairs at 5 kb. Based on these data, a SNP map of approximately 10 kb density should be adequate to screen this region for trait-marker associations. These data may actually underestimate the extent of LD in this region since they are based on phase-unknown genotypes from unrelated individuals. Determination of background LD is an important preliminary step in planning association studies of linked regions.

**Comparison of two micro-array based methods and Pyrosequencing for SNP genotyping.** *D.C. Chen<sup>1</sup>, J. Saarela<sup>1</sup>, I. Nuotio<sup>1</sup>, A. Jokiahho<sup>1</sup>, L. Peltonen<sup>1</sup>, A. Palotie<sup>2</sup>.* 1) Dept. Human Genetics and; 2) Pathology, UCLA, Los Angeles, CA.

Over one million sequence verified single nucleotide polymorphisms (SNPs) are currently deposited in the public databases. The challenge has now shifted from SNP identification to high-through SNP genotyping. Accordingly, efficient, flexible, and low cost SNP genotyping is a necessity for dense marker mapping as well as candidate gene association studies in complex diseases. We compared the performance of three SNP genotyping techniques based on primer extension: Affymetrix Genflex™ Tag array, an RNA polymerase extension based array and Pyrosequencing. A robust solid-phase single nucleotide extension reaction technique based on tritium detection was used as a reference method (Syvanen et al, Genomics, 1990). Fifteen SNPs, confirmed by sequencing, were selected on chromosome 17q. Using all four techniques including the reference method, 15 SNPs were studied in 96 related individuals. Without extensive optimization, we were able to genotype 10 out of 15 SNPs using the Genflex Tag array, 11 out of 15 SNPs using the RNA polymerase extension array, 11 out of 15 SNPs using Pyrosequencing and 12 out of 15 SNPs using the reference method. High GC content of the template and high tendency for sequencing primer to form dimer/hairpin loops are contributing factors to nonworking SNPs. The concordance rate of individual genotypes to the reference method varied among SNPs and ranged from 72% to 92 % for the Genflex™ Tag array, 83% to 97% for the RNA polymerase extension array and 87% to 99% for Pyrosequencing. The performance of different methods in the accuracy of genotyping was relatively similar. However, Pyrosequencing provided the highest proportion of successful SNP analyses and the highest precision for individual allele calls. It is likely that the single stranded template used both in the reference method and in Pyrosequencing is the major contributing factor for the better performance.

**LD across the *CYP2D6* region: implication for whole genome association scans in pharmacogenetic studies.** *L.K.*

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The clinical response of an individual to a medicine is dependent upon both genetic and environmental factors. *CYP2D6*, which metabolises approximately 30% of marketed medicines, has multiple genetic variants giving rise to different *CYP2D6* metabolising phenotypes. Association between the *CYP2D6* recessive poor metaboliser (PM) phenotype and genetic variation flanking the gene has been investigated. 33 single nucleotide polymorphisms (SNPs) with an average spacing of 26kb, were identified from an 870kb region on human chromosome 22 encompassing *CYP2D6*. Linkage disequilibrium and strong association (p values ranging from  $1.85 \times 10^{-3}$  to  $1.80 \times 10^{-33}$ ) were observed over 430kb between SNPs spanning *CYP2D6* and the PM phenotype. These findings support the strategy for the potential application of genome wide association scans in pharmacogenetic studies.

**Testing linkage disequilibrium in the presence of tight linkage and locus heterogeneity.** *J. Huang*<sup>1,2</sup>, *D. Wang*<sup>2</sup>, *Y. Jiang*<sup>4</sup>, *V.J. Vieland*<sup>1,3</sup>. 1) Statistics & Actuarial Sci, Univ Iowa, Iowa City, IA; 2) Dept of Biostatistics Div of Statistical Genetics, Univ of Iowa, Iowa City, IA; 3) Dept of Psychiatry, Univ of Iowa, Iowa City, IA; 4) Dept of Preventive Medicine and Epidemiology, Loyola Univ Medical Center.

Once a candidate gene has been discovered, it may be of interest to identify polymorphisms within the gene that display linkage disequilibrium (LD). This situation differs from one in which the interest is in detecting linkage via LD, since here the interest is in detecting LD under the assumption that there is tight linkage. We have developed a likelihood-based approach to assessing LD which conditions on tight linkage (recombination fraction (RF)=0). In addition, this approach makes use of all affected and unaffected individuals in the data (nuclear families); and it also allows explicitly for locus heterogeneity. The proposed likelihood has two parameters: an LD coefficient and a locus heterogeneity parameter. The latter parameter is the proportion of families in which the RF is 0, and the remaining proportion represents the families in which the RF is 0.5. A likelihood ratio test for LD in the presence of tight linkage and locus heterogeneity can be based on this likelihood. We have conducted simulation studies using recessive, dominant, additive and multiplicative models with various combination of disease and marker allele frequencies. The results show that the proposed test in general has better power than the TDT and a test proposed by Martin et al. (1997).

**Mutation screening and association study of *ATP10C* in Autism.** S. Kim<sup>1</sup>, L.B.K. Herzing<sup>2</sup>, J. Veenstra-VanderWeele<sup>1</sup>, C. Lord<sup>3</sup>, R. Courchesne<sup>4</sup>, B.L. Leventhal<sup>1,3,6</sup>, D.H. Ledbetter<sup>2</sup>, E. Courchesne<sup>4,5</sup>, E.H. Cook<sup>1,3,6</sup>. 1) Laboratory of Developmental Neuroscience, Department of Psychiatry MC3077, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Developmental Disorders Clinic, Child and Adolescent Psychiatry, Department of Psychiatry MC3077, University of Chicago, 5841 S Maryland Avenue, Chicago, IL; 4) Laboratory for Research on the Neuroscience of Autism, Children's Hospital Research Center, 8110 La Jolla Shores Drive, La Jolla, CA; 5) Department of Neurosciences, School of Medicine, University of California, San Diego, La Jolla, CA; 6) Department of Pediatrics MC3077, University of Chicago, 5841 S Maryland Avenue, Chicago, IL.

Autism is a complex genetic disorder. 15q11-q13 is of particular interest, because of previous reports of individuals with autism with predominantly maternal duplication or triplication of 15q11-q13. Linkage disequilibrium (LD) between polymorphisms in this region and autism has been also reported by several groups. Recently, a novel maternally expressed gene, *ATP10C*, was characterized and mapped to chromosome 15q11-q13 region, 200kb distal to *UBE3A*. It encodes a putative aminophospholipid translocase. Because of its physical location and maternal expression pattern in brain, *ATP10C* was considered to be a candidate gene for chromosome 15-associated autism. In an effort to find the genes responsible for autism in this chromosomal region, the coding and splicing regions of *ATP10C* were screened for sequence variants. Several polymorphic markers including five nonsynonymous SNPs were identified. To further investigate transmission disequilibrium between *ATP10C* and autism, a family-based association study was performed for 14 markers in 117 autism trios. Significant transmission disequilibrium was not found, suggesting *ATP10C* is unlikely to contribute to susceptibility to autism. However, the physiological role of the nonsynonymous SNPs and the functional implications of the SNPs identified from the 5' flanking region and the intron 2 splicing region may be evaluated in further studies.

**Tight association between diplotype configuration at NAT2 gene and adverse effects of sulfasalazine in patients with rheumatoid arthritis.** *Y. Kitamura*<sup>1</sup>, *E. Tanaka*<sup>2</sup>, *M. Saito*<sup>1</sup>, *N. Kamatani*<sup>1,2</sup>. 1) Division of Statistical Genetics, Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan; 2) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

**Purpose:** Part of the variations in adverse reactions to a drug can be explained by polymorphisms at the gene coding for drug-metabolizing enzyme. Arylamine-N-acetyltransferase 2 (NAT2) catabolizes sulfasalazine (SASP), a drug used for treatment of rheumatoid arthritis (RA). 7 SNPs have been identified in human NAT2 gene. Based on EM-algorithm, we estimated the *diplotype configuration* (i.e. a combination of 2 haplotypes) at NAT2 gene for Japanese subjects with RA treated with SASP and compared the *diplotype configurations* with outcomes of the patients.

**Materials and Methods:** Peripheral blood was withdrawn from 144 patients with RA who had been treated with SASP after informed consent was obtained. DNA was extracted and genotypes at 7 loci were determined by digestion with restriction enzymes. We have developed EM-algorithm-based LDSUPPORT software, which estimates posterior distribution over *diplotype configurations* for each subjects from genotypic data (Kitamura et al., 2001 manuscript submitted for publication).

**Results:** 6 haplotypes (wild-type; WT and 5 variants) were extracted for Japanese population. Estimated frequency for WT was 72.2%, while those for variant haplotypes were 19.1% (*GTTCAAG*:M2), 7.6% (*GTTGAA*:M3), 0.4% (*GCCCAAG*, *GTTGAG*) and 0.3% (*GCCTGGG*:M1). 16 patients were with adverse reactions to SASP. The patients with adverse effects had higher proportion of M2 (34.4%) and those without adverse effects had 17.2% ( $p < 0.05$ ). The patients without WT haplotype had higher incidence of adverse reactions than those with WT haplotype (62.5% vs 8.1%,  $p < 0.001$ ). In addition, the patients without WT haplotype had higher incidence of severe adverse reactions than those with WT haplotype (25.0% vs 1.5%,  $p < 0.005$ ).

**Conclusions:** Patients without WT haplotype had higher incidence of adverse reactions to SASP. Genotyping and subsequent estimation of *diplotype configuration* at NAT2 gene is useful to predicting the occurrence of adverse reactions to SASP.

**Determination of linkage disequilibrium patterns and mutation rate by high density SNP genotyping.** *D. Keen<sup>1</sup>, A. Ferrie<sup>2</sup>, J. Zielenski<sup>3</sup>, L.C. Tsui<sup>3</sup>, R. Parad<sup>4</sup>, R. Chung<sup>5</sup>, A. Menon<sup>6</sup>, M. Ramos-Arroyo<sup>7</sup>, F. Baas<sup>8</sup>, J. Kushner<sup>9</sup>, D. Housman<sup>1</sup>.* 1) MIT, Cambridge, MA; 2) Variagenics Inc, Cambridge, MA; 3) Hosp for Sick Children, Toronto, Canada; 4) Children's Hosp, Boston, MA; 5) Mass Gen Hosp, Boston, MA; 6) Univ of Cincinnati, OH; 7) Hosp Virgen del Camino, Pamplona, Spain; 8) Neurol Acad Med Ctr, Amsterdam, Netherlands; 9) Univ of Utah, Salt Lake City, UT.

In order to model the relationship between SNP haplotypes and functionally significant DNA sequence variation in humans we have investigated patterns of SNP distribution in CFTR (chromosome 7) and HFE (chromosome 6). We initially carried out a detailed sequence comparison over 5 kb surrounding the DF508 mutation of CFTR in 32 ethnically diverse individuals and 30 DF508 homozygotes. A number of SNP haplotypes are apparent in normal individuals, but there is no sequence variation among DF508 chromosomes. These data indicate that the DF508 mutation occurred on a specific haplotype and no recombination nor mutation has occurred within this 5 kb region since the original DF508 mutation arose. We extended the haplotype analysis to other CFTR mutations. Of particular interest, G542X, the second most frequent CFTR mutation in Caucasians, occurs on exactly the same haplotype background as DF508. Both DF508 and G542X have been estimated to originate 10-50,000 years ago based on microsatellite analysis. We carried out a detailed sequence comparison (65 kb) between the DF508 and G542X chromosomes. Both sequences were identical for more than 50 kb of this region with one exception, suggesting a mutation rate on the order of one base pair per  $10^8$  bases replicated per generation. The origins of framework haplotypes in the 65 kb region have been assessed by genotyping all SNPs in a number of ethnically divergent populations. The 65 kb sequenced region is divided by a recombinational hot spot identified through analysis of SNP haplotypes in normal individuals. A parallel analysis has been carried out in the HFE region of chromosome 6. Results of these studies have significant implications for the design and implementation of SNP based population association studies.



**Association Analysis of Autistic Disorder and SNPs in the chromosome 15 GABA<sub>A</sub> receptor subunit genes. M.M.**

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Autistic disorder (AutD) is a complex neurodevelopmental disorder with onset in early childhood (less than 36 months of age). It is characterized by abnormalities in social interactions and communication, as well as stereotypic and repetitive behaviors. Twin and family studies have shown evidence for a genetic component, but no clear mode of inheritance has been identified. Data from several genomic screens, as well as that from cytogenetic studies have indicated that a disease locus for AutD is located on the proximal portion of chromosome 15q. Contained within this region are genes for three of the subunits of the gamma-aminobutyric acid type A (GABA<sub>A</sub>) receptor. The neurotransmitter GABA mediates synaptic inhibition in the adult brain through its interaction with the GABA<sub>A</sub> receptors. These receptors are usually pentameric structures comprised of several different homologous subunits. The functional properties of a particular receptor are dependent upon the subunit composition. Two studies have shown evidence in support of linkage and association of AutD to the gene for one of these subunits: GABRB3. In order to further define the association between the chromosome 15q GABA<sub>A</sub> receptor subunit genes and AutD, we analyzed 16 single nucleotide polymorphisms (SNPs) located within GABRB3, GABRA5 and GABRG3, in 226 AutD families. The pedigree disequilibrium test (PDT) was used to test for linkage disequilibrium (LD). PDT results showed evidence for significant LD with two adjacent SNPs in the GABRG3 gene: exon5\_539T/C, P=0.02 and intron5\_687T/C, P=0.03. All other results were not significant (P>0.05). These data suggest that the GABRG3 gene contributes to genetic susceptibility in AutD and may represent the chromosome 15 AutD risk gene.

**Maximum likelihood estimator of two-biallelic markers haplotype frequencies, and its application in an LD exact test.** *S. Lissarrague, L. Essioux.* Statistical Genomics Dept, Genset Corp, Evry, France.

With the increased number of available markers and the decreasing costs of genotyping, large scale association studies are now feasible. One of the cornerstone parameter for performing such strategy is the linkage disequilibrium between neighboring markers, that requires estimation of two markers haplotypes frequencies (Reich et al. 2001). In association studies, haplotype analyses have become more and more popular because they can be more powerful than single markers associations (Fallin et al. 2001). Up to now, Estimation of haplotype frequencies are performed using iterative algorithms in order to find the most probable haplotypes frequencies given the genotypes data, such as the E-M algorithm (Excoffier and Slatkin, 1995). Those heuristics methods can be computationally intensive, and sometimes can lead to false solution due to local minimum of the likelihood function. Herein, we present An analytical maximum likelihood estimator of haplotypes frequencies in a set of unrelated individuals in the special case of two bi-allelic markers. The estimator is equivalent to the solution obtained by the E-M algorithm but is far less computationally intensive and always gives the true estimator. We then applied this estimator to construct an optimized exact likelihood ratio test for Linkage equilibrium which is more precise and less computer intensive than the traditional Monte-Carlo approach.

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**Linkage disequilibrium at *PPARG* and other genes assessed with dense sets of SNPs.** *J.N. Hirschhorn<sup>1,2,3</sup>, D. Altshuler<sup>1,4</sup>, M.J. Daly<sup>1</sup>, C.M. Lindgren<sup>1,5</sup>, N.P. Burt<sup>1</sup>, M. Loomer<sup>1</sup>, A. Villapakkam<sup>1</sup>, S. Bolk<sup>1</sup>, C.J. O'Donnell<sup>6</sup>, J.D. Crews<sup>1</sup>, L.C. Groop<sup>5</sup>, E.S. Lander<sup>1,7</sup>.* 1) Center for Genome Research, Whitehead Inst./MIT, Cambridge, MA; 2) Divs. of Endocrinology and Genetics, Children's Hosp., Boston, MA; 3) Depts. of Pediatrics and Genetics, Harvard Med. School, Boston, MA; 4) Depts. of Genetics and Medicine, Harvard Med. School and Mass. General Hosp., Boston, MA; 5) Div. of Endocrinology, Lund Univ., Malmö, Sweden; 6) NHLBI/Framingham Heart Study, Framingham, MA; 7) Dept. of Biology, MIT, Cambridge, MA.

Significant linkage disequilibrium (LD) around disease alleles would enable the use of nearby genetic markers to detect association to disease. Thus, measuring LD near disease alleles and within genes is relevant for the prospects of mapping disease genes.

We identified 17 informative single nucleotide polymorphisms (SNPs) in 19 kb surrounding a known disease allele, *PPARG* Pro12Ala (associated with type 2 diabetes). Typing these SNPs in >300 Caucasian trios revealed strong LD: there are only 4 major haplotypes in this region, 3 with the ancestral proline allele and 1 bearing the alanine allele. Additional markers showed LD at distances of over 80 kb. Remarkably, 7 of the 17 SNPs were nearly perfect proxies for Pro12Ala, demonstrating that the resolution limit of genetic mapping at this locus has been reached in this Caucasian population. We next studied LD in 33 additional genes, chosen for relevance to cardiovascular traits. For all 804 publicly available SNPs within the 33 genes or in the 5 kb on either flank, we attempted assay design and genotyping in 92 individuals from 12 CEPH families. Consistent with results from random genomic regions (Bolk et al. abstract), LD varied from gene to gene, but strong LD was seen over entire genes. For such genes, all common haplotypes could be marked by typing only a few SNPs. These results suggest that LD-based association may well be a useful approach to mapping common disease alleles. However, prior explicit determination of LD structure may be required to maximize the efficiency of such an approach.

Program Nr: 1998 from the 2001 ASHG Annual Meeting

**Study of linkage disequilibrium between human dopamine D1 receptor (DRD1) gene and bipolar disorder.** X. Ni, J.M. Trakalo, E. Mundo, F. Macciardi, L. Lee, J.L. Kennedy. Neurogenetics Section, Ctr Addiction & Mental Health, Toronto, Ontario, Canada.

Abstract: Based on the dopamine hypothesis, the dopamine D1 receptor (DRD1) gene is considered to be a good candidate gene for bipolar (BP) disorder. In the present study, we analysed three polymorphisms of the DRD1 gene, -800T/C, -48A/G and 1403T/C in 286 BP disorder trios. Both the extended transmission disequilibrium test (ETDT) and haplotype TDT were performed on the genotype data to test the presence of linkage disequilibrium between DRD1 gene and bipolar disorder. With the same test we also calculated the maternal transmission or paternal transmission for each allele. Though no association was found for each individual polymorphism, there was a significant association between DRD1 gene and BP disorder for haplotype TDT analysis ( $X^2=21.307$ ,  $df=7$ ,  $p=0.0028$ ). These results may indicate that the DRD1 gene plays a role in the etiology of bipolar disorder. Keywords: bipolar disorder, genetics, linkage, transmission disequilibrium test, haplotype transmission disequilibrium test, dopamine D1 receptor gene.

**Type 2 diabetes and calpain-10 gene polymorphisms in Samoans.** *H-J. Tsai<sup>1</sup>, G. Sun<sup>2</sup>, D.E. Weeks<sup>1</sup>, S.T. McGarvey<sup>3</sup>, R. Kaushal<sup>2</sup>, M. Wolujewicz<sup>2</sup>, R. Deka<sup>2</sup>.* 1) Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Environmental Health, University of Cincinnati, Cincinnati, OH; 3) International Health Institute, Brown University, Providence, RI.

Although genome wide scans have identified several potential chromosomal susceptibility regions in several human populations, finding a causative gene for type 2 diabetes has remained an enigma. Horikawa et al. (Nat Genet. 2000. 26:163-175) reported a novel gene, calpain-10 (*CAPN10*), located in a previously identified region on chromosome 2q37.3, as a putative type 2 diabetes susceptibility gene. Three single nucleotide polymorphisms (UCSNP43, UCSNP19, UCSNP63) were shown to be involved in increased risk to the disease among Mexican Americans. We have tested the association of these three polymorphisms with type 2 diabetes among the Samoans of Polynesia, who have a very high prevalence of the disease. In the US territory of American Samoa, prevalence is 25% and 15% in men and women, respectively, compared to 3% and 5% in men and women, respectively, from the independent country of Samoa. In our study sample consisting of 96 controls and 295 affected sib pairs in 174 families recruited in American Samoa, we failed to detect any association between cases and controls in allele frequencies, haplotype frequencies or haplotype combinations of SNP43, 19 and 63. Also, there was no evidence of linkage in our data in the region of chromosome 2 containing these SNPs. Three plausible scenarios could explain these observations: the underlying biological mechanism is too complex and needs further research; *CAPN10* is a susceptibility gene in some particular ethnic groups; our study lacks power to detect the effects of *CAPN10* polymorphisms. With regard to power, however, our number of families (174) is comparable to the number (170) in the original Horikawa et al. study. Supported by NIH grants AG09375, HL52611, DK55406 and DK59642.

**Linkage Disequilibrium Mapping of a Putative Type 2 Diabetes Locus (1q21-q23) using Pooled DNA.** *J.K. Wolford, S. Kobes, R.L. Hanson, C. Bogardus, M. Prochazka.* NIDDK/PECRB, NIH, Phoenix, AZ.

We have previously reported linkage of type 2 diabetes mellitus to Chromosome 1q21-q23 in Pima Indians and are presently fine-mapping this putative diabetes susceptibility locus by linkage disequilibrium using densely spaced single nucleotide polymorphisms (SNPs) to complement analyses of known positional candidate genes in this region. From public databases and through our own work we have identified over 3500 SNPs spanning a ~20 cM region of the linkage interval. We are currently screening these markers for differences in allelic frequency in 2 pools consisting of 150 diabetic and 150 non-diabetic Pimas (none are first degree relatives). Using the MassArray technology of Sequenom, Inc, we have to date assayed 1029 potential SNPs in these pools, including 90 SNPs which we identified by sequencing or denaturing high performance liquid chromatography and 939 obtained from dbSNP (<http://www.ncbi.nlm.nih.gov/SNP>). Of these, 522 SNPs (50.7%) were informative (allelic frequency >10%), 151 SNPs (14.7%) were non-informative (allelic frequency ≤10%), and 356 (34.6%) SNPs were non-polymorphic in our samples. Further analysis of the raw spectra for each assay indicated 339 SNPs (32.9 %) with either a high baseline or multiple peaks and consequently, we are repeating these assays. Average density is 1 informative SNP per 41.3 (±5.4) kb and the largest gap is 1.4 Mb; gaps greater than 50 kb (N=28) are being filled with additional SNPs. Preliminary analyses have identified 7 SNPs (4 of which are clustered within a 1 Mb region) with significantly different ( $P < 10^{-3}$ ) allele frequencies in the diabetic vs. non-diabetic pools and we are presently genotyping these in individual samples.

**Analysis of extent of linkage disequilibrium using more than a thousand of SNPs throughout the genome in Japanese population.** *T. Tsunoda*<sup>1</sup>, *R. Yamada*<sup>1</sup>, *M. Yamaguchi*<sup>1</sup>, *H. Kawakami*<sup>2</sup>, *Y. Ohnishi*<sup>1</sup>, *A. Sekine*<sup>1</sup>, *T. Tanaka*<sup>1</sup>, *Y. Nakamura*<sup>1,2</sup>. 1) SNP Research Center, RIKEN, Tokyo, Japan; 2) Institute of Medical Science, University of Tokyo, Tokyo, Japan.

Recent progress in whole-genome discovery and high throughput genotyping technology of single nucleotide polymorphisms (SNPs) has brought much possibility to detect association between SNP markers and common complex genetic traits with linkage disequilibrium (LD) mapping strategy. The sensitivity to locate regions in association and the efficiency to pinpoint genuine locus in the associated regions of LD mapping method largely depend on the extent of LD. The extent of LD is expected to vary among ethnic groups and regions in the genome. We evaluated for the first time the extent of LD throughout the genome excluding sex chromosomes with 1,227 SNPs genotype data from more than 752 of Japanese. The SNPs genotyped located on exons, surrounding introns and 5 flanking region of known or predicted-in-silico genes throughout the genome. More than 752 Japanese individuals were genotyped for each SNP. From all the SNPs assayed, only the SNPs with their minor allele frequency more than 0.2 were included for analysis of LD to exclude pseudo-LD. Every SNP was mapped on a complete contig from NCBI. LD index,  $D$ , between every pair of two SNPs on the same contig was calculated using EM-algorithm. The result of the large-scale analysis showed that the average  $D$  in a Japanese population dropped below 0.5 at 40kb, which was between Utah population (60kb) and Nigerian population (<5kb), and the fraction of  $D > 0.5$  was 25% at 90-110kb. Our result suggested that the use of Japanese population will enable more efficient pinpointing of genuine loci than the case using the Utah population.

**Multilocus measures for linkage disequilibrium.** *J.P. Rice, N.L. Saccone, P.-Y. Kwok.* Washington University School of Medicine, St. Louis, MO.

The utility of genetic linkage maps has been in part due to the existence of a metric (map distance), which allows the map locations of markers to determine all possible recombination fractions between markers, and allows the placement of new markers or disease genes. In contrast, a marker may lie between two markers in strong disequilibrium, and yet be in equilibrium with both. Mechanisms such as drift, mutation and population admixture may lead to patterns inconsistent with linkage disequilibrium (LD) that is solely due to recombination. The available set of Single Nucleotide Polymorphisms (SNPs) has grown at a rapid pace, although most analyses have been descriptive and based on pair-wise comparisons.

We develop a set of conditional LD measures for a set of markers (X,Y,Z) where the usual D and D-prime statistics are computed between X and Z conditional on the genotype at the intervening marker Y. We note the changes between generations in the  $2 \times 2 \times 2$  table of frequencies if LD is due to recombination, and present simple examples in which the conditional measures can be computed algebraically. In this setting the conditional measures are small even after one generation of random mating.

We examine 6 SNPs on the X chromosome within a 500 kb region on Xq28. Three SNPs (Xq3449-1, Xq3274-1, and Xq1452-1) showed D-primes of 0.66 and 0.36 between consecutive pairs, with conditional values of 0.06. If these three were used as a "map" in this region, the other 3 SNPs showed significant LD with at least one of them. Two of the extra SNPs had D-prime values of 1 and -1 with one of the SNPs in the map. The map thus captures the LD information in this region.

From the limited data available so far, it is clear that some sets of SNPs show irregular patterns for these conditional measures. However, given the number of SNPs available in any region, it is likely that a subset can be chosen with moderate LD between each pair and minimal conditional LD. It remains to be seen whether such a map would be optimal for the detection of disease genes through LD mapping.



**Strong linkage disequilibrium among the CYP2C subtypes in Japanese population.** *M. Saito*<sup>1</sup>, *Y. Kitamura*<sup>1</sup>, *T. Kubota*<sup>2</sup>, *T. Iga*<sup>2</sup>, *N. Kamatani*<sup>1</sup>. 1) Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan; 2) Department of Pharmacy, University of Tokyo Hospital, Tokyo, Japan.

**Purpose:** Proteins that constitute cytochrome P450 (CYP) are important for oxidative degradation of various foreign compounds. They are coded for by a family of CYP-related genes among which CYP2C subfamily plays an important role. The 3 genes, CYP2C18, CYP2C19, and CYP2C9 all of which belong to the CYP2C subfamily have been mapped to the same region of chromosome 10. We examined the linkage disequilibrium (LD) in this region to investigate the nature of this gene family.

**Materials and Methods:** The genotypic data of polymorphism at 4 sites in CYP2C18 and CYP2C19 have been published by Kubota *et al.* (Kubota *et al.*, 1998, *Biochemical Pharmacology*, **55**, 2039-2042). These and additional data for a SNP of CYP2C9 were used for the estimation of haplotype frequencies and the calculation of posterior probability of diplotype configuration for each of the subjects using LDSUPPORT, an expectation-maximization (EM)-based maximum likelihood estimation software.

**Results:** Although the 3 genes are scattered over at least 100 kb region (0.25 cM), there was a strong LD between SNPs in the 3 genes. Between the SNPs of CYP2C18 and CYP2C19, the LD was virtually complete. The estimated frequency for wild type, T (T<sub>478</sub>->C in CYP2C18) - T (T<sub>204</sub>->A in CYP2C18) - G (G<sub>636</sub>->A in CYP2C19) - G (G<sub>681</sub>->A in CYP2C19) - A (A<sub>1075</sub>->C in CYP2C9), was 53% (34% in the assumption of no LD), and those for other major variant haplotypes were 31% (CTGAA, 7.4% in no LD) and 13% (TAAGA, 0.8% in no LD).

**Conclusions:** The genotypic data in the genomic region containing 3 genes belonging to a subfamily of CYP2C showed an evidence of strong LD. The function of CYP2C which includes the protection of the organism from unpredictable attacks of foreign compounds is somewhat similar to that of HLA (human leukocyte antigen) in which a strong LD is reported. The strong LD among the genes of CYP2C may have been maintained during the evolution of a defense mechanism.

**A Pakistani family with autosomal recessive non-syndromic hearing loss linked to the DFNB7/11 locus. R.**

*Abbasi<sup>1</sup>, S.A. Shami<sup>1</sup>, R. Qamar<sup>2</sup>, Q. Ayub<sup>2</sup>, S. Khaliq<sup>2</sup>.* 1) Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan; 2) Biomedical and Genetic Engineering Division, Dr. A. Q. Khan Research Laboratories, Islamabad, Pakistan.

Autosomal recessive non-syndromic hearing loss is the most common form of severe inherited childhood deafness. We studied nineteen members of a large consanguineous Pakistani family affected with autosomal recessive, non-syndromic, prelingual profound hearing loss. Previously reported loci for autosomal recessive non-syndromic hearing loss were excluded, with the exception of marker D9S301 on chromosome 9q21.11. All the affected individuals were homozygous for this marker and provided a strong evidence of linkage with the disease phenotype with a maximum LOD score of 5.70. The combined DFNB7/DFNB11 locus has been previously mapped to chromosome 9q13-q21 in separate families of Indian and Israeli origin. This locus span an interval of approximately 1cM bounded by the markers D9S1806 and D9S769. Two genes expressed in the cochlea (ZNF216 and TMEM2) that map to this region have already been excluded for disease association. There are several putative genes spanning this region in the database that could be associated with the disease phenotype.

**Quantitative trait loci underlying variation in blood cell concentrations.** *D.M. Evans<sup>1</sup>, G. Zhu<sup>1</sup>, I.H. Frazer<sup>2</sup>, N.G. Martin<sup>1</sup>.* 1) Genetic Epidemiology, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; 2) Centre for Immunology and Cancer Research, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia.

Blood cell concentrations remain stable across time in healthy individuals, yet vary widely between different individuals. We and others have demonstrated that part of this large interindividual variation is the result of genetic factors. The aim of the present study was to identify quantitative trait loci responsible for this variation. Complete blood cell counts (i.e. hemoglobin, red cell count, mean cell volume, platelet, neutrophil, eosinophil, basophil, monocyte and lymphocyte counts) and lymphocyte subsets analyses (CD4+ T cell, CD8+ T cell, B cell and natural killer cell counts) were performed in 232 pairs of dizygotic twins at twelve, fourteen and sixteen years of age. A genome scan consisting of 400 highly polymorphic markers was performed on each twin's DNA. Identity by descent probabilities for each twin pair were calculated using Genehunter 2.0 and this information was incorporated into a variety of structural equation models for variance components linkage analysis.

Univariate linkage analyses identified promising regions of genetic linkage for eosinophil count, platelet count and CD4-CD8 ratio. These linkages were present at similar chromosomal regions at age twelve, fourteen and sixteen in the case of platelets and eosinophils, and at twelve and fourteen years only in the case of CD4-CD8 ratio. Analyzing each cell count longitudinally using multivariate structural equation models increased the power to detect linkage at some of these markers. The genomic region of interest for platelets mapped closely to a gene which has been implicated in platelet formation, albeit in a minor role. The regions of interest for eosinophils and CD4-CD8 ratio, however, did not contain any obvious candidate genes. We are currently genotyping an additional 200 dizygotic pairs in an attempt to increase the evidence for linkage at these loci.

**Evidence of an Epistatic Interaction in a Genetic Linkage Study of Autism.** *M.F. Dowd, R. Mankoski, S. Santangelo, S. Folstein.* CLSA, New England Medical Center, Boston, MA.

Epistatic interactions were investigated in the followup data from 86 families in the Collaborative Linkage Study of Autism. Followup data were available for selected regions on chromosomes 1, 2, 4, 6, 7, 8, 11, 13, 14, 15, 16, 19, and X. Although it is known that genes involved in epistatic interactions can be detected by using single-locus linkage methods, we are more interested here in where the interactions are taking place, i.e., which specific pairs of loci are involved. We first considered correlations between family NPL scores from various locations along the genome and produced a short list of candidate locus-pairs, which we then analyzed using the program GENEHUNTER-TWOLOCUS. A wide range of epistatic as well as heterogeneity models were considered. We found significant evidence of an epistatic interaction between loci that are located at D1S1653 and D13S217, where parametric two-trait-locus analysis yields a MOD-score greater than 6. One of the most interesting aspects of this result is that the signal near D1S1653 under one-trait-locus analysis was not particularly strong and the one near D13S217 was marginal. This appears to re-confirm the finding by Schork et al., (1993) that parametric two-trait-locus analysis can reach higher power to detect linkage than its single-trait-locus counterpart.

**Localization of Migraine Susceptibility Genes to Chromosomes 1q, 19p and Xq.** *L.R. Griffiths<sup>1</sup>, R.A. Lea<sup>1</sup>, R.P. Curtain<sup>1</sup>, S. Quinlan<sup>1</sup>, J. Chappell<sup>1</sup>, J.C. MacMillan<sup>2</sup>.* 1) Genomics Research Centre, Griffith University Gold Coast; 2) Queensland Clinical Genetics Service, Royal Childrens Hospital, Brisbane, Queensland, Australia.

Studies in our laboratory have been directed towards identifying genes involved in the complex neurological disorder, migraine. These studies have localized migraine gene components on chromosomes 19p13(1), Xq24-28(2) and more recently on 1q(3). These studies have utilized large multigenerational migraine pedigrees of sufficient power for significant independent results, with one family (MF1) showing both cosegregation and significant allele sharing (NPL=6.64, P=0.0026) for markers located across a 12.6cM region of 19p13, and two families (MF7 and MF14) showing significant excess allele sharing to Xq markers with a max LOD score of 2.388 (P=0.0005). More recently we have identified two additional families showing significant excess allele sharing to the same Xq24-28 region. Also of interest, recent studies have indicated that MF14, one of the families showing allele sharing on Xq, also shows significant allele sharing (P=0.001) to 1q markers, indicating that the disorder in a single large affected pedigree may involve more than one and possibly interacting gene components. Results from 82 independent migraine pedigrees also show significant allele sharing (P=0.013) and allele transmission distortion (P=0.021) to this region of 1q. Overall, these 1q studies resulted in a max LOD score of 3.11 (P=0.00007) obtained for the D1S249 marker located within the 1q31 genomic region. Hence our results indicate that migraine is a complex polygenic disorder, involving multiple and possibly interacting gene loci.

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**A Complete Set of Constraints and Parameterisation of Two-Locus Affected Sib-Pair IBD Probabilities. O.**

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To increase power in the analysis of affected sib-pair (ASP) data, estimation of the identity by descent (IBD) probabilities is often constrained to the possible triangle (Holmans 1993). We extend previously derived constraints (Cordell et al. 1995) on the nine joint IBD probabilities at two unlinked loci to a set of nine linearly independent inequalities which constitute the natural two-locus analogue of the triangle.

The constraints follow from similar constraints on the sixteen sex-specific IBD probabilities (allowing parental imprinting), that are satisfied under two types of general models. Under one model, including no population genetic assumptions, restrictions on the penetrances are needed. These restrictions are, however, satisfied by e.g. the multiplicative, additive and heterogeneity multi-locus penetrance models. The other model includes Hardy-Weinberg equilibrium (HWE), random mating (RM) and linkage equilibrium (LE), but no penetrance assumptions.

Two-locus ASP IBD probabilities satisfying the constraints can be conveniently expressed in terms of a set of parameters that are unconstrained, except for being positive and summing to one. Under HWE, RM and LE the parameters may be interpreted in terms of genetic variance components, population prevalence and sibling recurrence risk (cf. Cordell et al. 2000). Unconstrained parameters are advantageous in estimation and derivation of statistical tests. Furthermore the parameterisation readily extends to more than two loci and is promising as a means for better understanding the relationship between gene interaction (defined by multi-locus penetrances) and joint IBD proportions.

**Linkage analysis of a family with Late Onset Atrophic Macular Degeneration and exclusion of candidate loci.** *R. Ayyagari<sup>1</sup>, L.E. Kakuk<sup>1</sup>, S. Atkins<sup>1</sup>, P.A. Sieving<sup>1,2</sup>*. 1) Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI; 2) National Eye Institute, NIH, Bethesda, MD.

We identified a large pedigree with autosomal dominant late onset atrophic macular degeneration. Affected individuals initially showed perifoveal RPE atrophy that evolved to central areolar chorioretinal atrophy with age. Ganzfeld ERG showed normal rod and cone function despite marked reduced visual acuities of 20/30 to 20/400. Visual symptoms became apparent around age 40, with strong penetrance. Linkage analysis excluded the known macular degeneration loci, including Stargardt's macular degeneration, Sorsby's fundus dystrophy, Best's macular degeneration, Stargardt-like macular degeneration (STGD3), STGD4, North Carolina macular degeneration, progressive bifocal chorioretinal atrophy, Doyn's Honey Comb Dystrophy, Cone Rod Dystrophy 3 (CORD3), CORD7, RDS/Peripherin. We have also completed scanning 25% of the genome for linkage using 10-cM marker panel with out identifying a region with positive LOD scores. We are in the process of genome wide scanning to map the disease locus in this family. The phenotype observed in this family overlaps with other forms of atrophic macular degenerations including age-related macular degeneration (AMD). Based on our current results it is likely that the disease locus in this family is associated with a novel gene that has not previously been shown to be involved in macular degeneration.

**A new form of Autosomal Dominant late onset Spinal Muscular Atrophy.** *A.L.F. Bernardino<sup>1</sup>, A. Richieri-Costa<sup>2</sup>, M.R. Passos-Bueno<sup>1</sup>, A.M.P. Cerqueira<sup>1</sup>, M. Zatz<sup>1</sup>.* 1) Centro de Estudos do Genoma Humano, Univ de São Paulo, São Paulo, São Paulo, Brazil; 2) Centro de Reabilitação em Anomalias Craniofaciais, Univ de São Paulo, São Paulo, Brasil.

A large family with at least 40 affected members by an Autosomal Dominant (AD) late onset Spinal Muscular Atrophy was reported by Finkel et al.; (1962) and Richieri-Costa et al.; (1981). The manifestations are slow loss of muscle strength and progressive proximal atrophy (which starts in the lower limbs and progresses to the upper limbs) hipoactive or absent tendinous reflexes and generalized fasciculation. Through linkage analysis we have excluded the 6 genes responsible for AD Limb-Girdle Muscular Dystrophies, 2 genes responsible for AD Spinal Muscular Atrophy and 5 genes responsible for AD Charcot-Marie-Tooth Neuropathy. In an attempt to identify the disease-causing gene we are currently performing a genome wide scan using microsatellite markers at an average distance of 10cM. So far, fourteen chromosomes were screened and excluded (1, 3, 5, 7, 9, 13, 14, 15, 16, 17, 19, 20, 21 and 22) and we are continuing the analysis for the other chromosomes. Supported by: FAPESP-CEPID, PRONEX, CNPq.



**A first locus for non syndromic autosomal recessive optic atrophy (OAR1).** *F. BARBET<sup>1</sup>, S. GERBER<sup>1</sup>, J.-M. ROZET<sup>1</sup>, I. PERRAULT<sup>1</sup>, D. DUCROQ<sup>1</sup>, S. HANEIN<sup>1</sup>, J.-L. DUFIER<sup>2</sup>, A. MUNNICH<sup>1</sup>, J. KAPLAN<sup>1</sup>.* 1) Laboratoire de Recherches sur les Handicaps Genetiques de l'Enfant, INSERM U393, Hopital des Enfants Malades, Paris Cedex 15, FRANCE; 2) Service d'Ophtalmologie, Hopital Necker, Paris, FRANCE.

In contrast with dominant optic atrophies, in which the optic atrophy is usually an isolated event, autosomal recessive optic atrophies (ROA) are frequently multisystem diseases. Yet, we ascertained 7 unrelated multiplex families in which the optic atrophy is not associated with any other symptom. The goal of this study was to localize one or more genes responsible for this very uncommon visual disorder. Putative linkage with the dominant optic atrophy gene (OPA1) on chromosome 3q28 was first excluded in all seven families. Subsequently, a wide genome search for homozygosity was undertaken in 2/7 multiplex and consanguineous families affected with non syndromic OAR. The 382 pairs of fluorescent oligonucleotides of the Genescan Linkage Mapping Set, Version II (Perkin Elmer Cetus) have been studied under conditions recommended by the manufacturer. Amplified fragments were electrophoresed and analyzed on an automatic sequencer (ABI 377). The polymorphic markers have an average spacing of 10 cM throughout the genome. Linkage analyses were performed using M-LINK and LINKMAP of the 5.1 version of the Linkage program. We found evidence for homozygosity in one of the two families studied here. In this family originating from France, four affected children and one healthy child were born from second cousin parents. All 4 affected patients were found to be homozygote for polymorphic markers which were informative in their parents. Pairwise lod-score values calculated in this family gave a maximum lod-score of 3.51 at  $Q = 0$  with the most informative marker. This locus has been excluded by linkage analyses in the six other families. In conclusion, we report here the first mapping of isolated autosomal recessive optic atrophy in 1/7 multiplex families. These data strongly suggest the genetic heterogeneity of this rare condition.

**Affected Sib Pair Tests in Inbred Populations.** *W. Liu, B.S. Weir.* Bioinformatics Program, North Carolina State University, Raleigh, NC.

Affected-sib-pair (ASP) methods study the possible genetic linkage between a putative disease susceptibility locus and marker loci by demonstration within families of nonrandom segregation of parental alleles in affected children. Some ASP tests study the proportions of identical by descent (IBD) marker alleles, others study the identical by state (IBS) marker alleles. Both IBD and IBS tests generally assume that parents are unrelated and not inbred. If there is relatedness and inbreeding, even if only because of evolutionary history, the expected proportions of both IBD and IBS marker alleles are increased. If the usual ASP methods are applied in an inbred population, therefore, high false positive rates may be obtained. We have modified two identity by state test statistics (t-test and chi-square test) of Lange to allow inbreeding in the population. In both tests, the expected parental mating type frequencies were modified. We evaluated the power and false positive rates of both tests under three disease model using simulated data and compared them with those obtained from the usual tests. When the population inbreeding coefficient is large, both the false positive rates and power are reduced when the modified test statistic were applied, although power remained high under a recessive disease model. Our modified IBS tests could provide a good screen when the disease is known to be recessive.

**Fine-mapping of the mouse *ApoB regulator gene 1 (Abrig1)* locus, which regulates plasma apoB levels, to a 2-cM segment of chromosome 6.** L.S. Huang, T.L. Lee, M. McQuade, N. Luo, P.W. Lau, C. Ko, S.C. Chua, Jr. Dept Medicine, Columbia Univ, New York, NY.

Elevated plasma apolipoprotein B (apoB) is a strong predictor of atherosclerosis and coronary heart disease. We have previously shown that *ApoB regulator genes (Abrgs)* are associated with plasma apoB levels in congenic human apoB transgenic (HuBTg) mouse strains. The gene products likely regulate the levels of apoB via hepatic apoB secretion rates. Using F2 and N2 crosses between C57BL/6 (B6-high apoB) and 129/Sv (129-low apoB) strains, we mapped *Abrig1* and *Abrig2* to mouse chromosomes 6 and 4, respectively. Interval-specific congenic strains for *Abrig1* were generated by selecting animals heterozygous for the two markers which define the interval: D6Mit55 and D6Mit199. Incipient congenics (N3-N7) preserved the phenotype of *Abrig1*: Plasma human apoB levels in B6 HuBTg mice containing one copy of 129 allele of *Abrig1* were always lower (30%) than those in B6 HuBTg mice. Both known and new markers spanning the critical interval were used to genotype N4 mice (n=843). The genetic distances among critical markers are as follows: D6Mit55 (49.7 cM) -D6Mit44 (51.5 cM) -D6CU39 (53.5 cM) -D6CU14 (54.0 cM) -D6Mit344 (55.7 cM) -D6Mit368 (56.4 cM) -D6Mit199 (68.0 cM). Reference distances derived from the MGI Database are underlined. Results from N4 recombinants showed that *Abrig1* is within a 2-cM segment flanked by the D6Mit44 and D6CU39 markers. Genetic analysis of F2 and N2 mice (n=88 total) derived from another cross (B6xC3H) also confirmed the presence (LOD=6.2 at D6Mit150, 51.0 cM) of *Abrig* variants between the B6 and C3H strains. Identification of mouse *Abrig* genes may prove useful for finding human orthologues that regulate plasma apoB levels.

**A quantitative trait locus (QTL) on chromosome 5 is associated with plasma apoB responsiveness to fish oil in human apoB transgenic mouse strains.** *C. Ko, S. Kim, W.M. Wu, V. Thassanapaff, S.C. Chua, Jr., L.S. Huang.* Dept Medicine, Columbia University, New York, NY.

An elevation of plasma apoB level is associated with a high risk of atherosclerosis and coronary heart disease. Utilizing a human apoB transgenic (HuBTg) mouse model, we have shown that the responsiveness of plasma apoB levels to fish oil (FO) feeding is genetically regulated. Upon 2 weeks of FO feeding, the C57BL/6 (B6) strain showed a low response in the apoB level, while the FVBxB6 F1 mice showed a high response. To identify genetic factors regulating this responsiveness, F1 HuBTg mice were backcrossed to B6 wild-type mice to generate N2 offspring. Male N2 HuBTg mice (n=150) were fed a chow diet until 8 weeks of age and switched to FO for 2 weeks. Animals were bled before (baseline) and 2 weeks after FO feeding. Plasma human apoB levels were assessed at both time points. Male N2 mice were subjected to a total genome scan using microsatellite markers. Linkages between plasma apoB levels and genotypes were assessed using Map Manager QT program. Analysis of the results derived from 53 markers revealed a significant linkage (LOD score =3.1) between marker D5Mit43 (83.0 cM, chromosome 5) and plasma apoB levels after 2 weeks of FO feeding. Homozygotes for the B6 allele (BB) had significantly lower plasma apoB levels than heterozygotes (FB) ( $85\pm 13$  vs.  $95\pm 16$  mg/dl,  $p=0.0002$ ). No significant differences were found in the baseline plasma apoB levels between both groups of animals (BB vs. FB:  $81\pm 10$  vs.  $82\pm 10$  mg/dl). These results showed that homozygotes had a low response (DapoB= $3\pm 14$  mg/dl), whereas heterozygotes had a high response (DapoB= $13\pm 16$  mg/dl) to FO. In summary, we have identified a QTL on chromosome 5 which regulates the responsiveness of plasma apoB levels at a specific time interval following fish oil feeding. Further studies of this QTL may shed light on the mechanisms regulating plasma apoB responsiveness to fish oil.

**A genome wide scan reveals a putative novel locus for primary ciliary dyskinesia (PCD) in the Faeroe Island population.** *D. Jeganathan<sup>1</sup>, M. Meeks<sup>1</sup>, C. Gehrig<sup>2</sup>, A.J. Walne<sup>1</sup>, M.G. Dunn<sup>1</sup>, H. Bisgaard<sup>3</sup>, J.L. Blouin<sup>2</sup>, R.M. Gardiner<sup>1</sup>, E.M.K. Chung<sup>1</sup>, H.M. Mitchison<sup>1</sup>.* 1) Department of Paediatrics and Child Health, RFUCMS, University College London, UK; 2) Division of Medical Genetics, Geneva University Cantonal Hospital and School of Medicine, CMU, Geneva, Switzerland; 3) Department of Paediatrics, CUH, Copenhagen, Denmark.

PCD is an autosomal recessive disorder (incidence of 1:20,000 live births) characterised by respiratory tract infections, sinusitis, bronchiectasis and subfertility. The clinical phenotype results from dysmotility of the cilia which is associated with a variety of different structural abnormalities. About 50% of patients exhibit laterality defects (Kartagener syndrome). To identify a gene locus for PCD, we studied 4 nuclear families (7 affecteds and 14 unaffecteds) from the isolated Faeroe Island population. The Faeroe Islands have a small population of 45,000, established relatively recently (approximately 1100 years ago). Patients have an outer dynein arm ciliary defect and none display situs inversus. A genome wide scan identified a region consistent with linkage on chromosome 16. High density haplotype analysis refined the region of linkage to a 23cM interval on chromosome 16p12 between D16S3041 and D16S409. Linkage analysis at this interval using GENEHUNTER gave a maximum multipoint lodscore of 2.7 between D16S412 and D16S3093. Assuming a single ancestral mutation accounts for all patients in this isolated population, the identification of linkage disequilibrium by additional marker typing should further refine the critical interval. The narrowed region between D16S412 and D16S3093 spans a distance of about 5.5Mb, approximately of which 50% is available as finished genomic sequence. We are constructing a transcript map for the isolation of positional candidate genes. An axonemal dynein heavy chain, DNAH3, maps to marker D16S417 within our narrowed region (Maiti et al., *Eur J Hum Genet* 8:923, 2000). This presents an excellent candidate gene for PCD in our patients. The identification of intragenic variants within DNAH3 has extended the linkage analysis. Currently further characterisation and mutation screening of this gene is underway.

Program Nr: 2016 from the 2001 ASHG Annual Meeting

**The Ising Model in Physics and Statistical Genetics.** *J. Majewski<sup>1</sup>, H. Li<sup>2</sup>, J. Ott<sup>1</sup>*. 1) Laboratory of Statistical Genetics, Rockefeller Univ, New York, NY; 2) Department of Biochemistry and Biophysics, University of California, San Francisco.

Interdisciplinary communication is becoming a crucial component of the present scientific environment. Theoretical models developed in diverse disciplines may often be successfully employed in solving seemingly unrelated problems that can however be reduced to similar mathematical formulation. The Ising model has been proposed in statistical physics as a simplified model for analyzing magnetic interactions and structure of ferromagnetic substances. Here, we present an application of the one-dimensional, linear Ising model to affected sib pair (ASP) analysis in genetics. By analyzing simulated genetic data, we show that the simplified Ising model with only nearest neighbor interactions between genetic markers has statistical properties comparable to much more complex genetic analysis algorithms, such as those implemented in the Allegro and Mapmaker-Sibs programs. We also adapt the model to include epistatic interactions and demonstrate its usefulness in detecting modifier loci with weak individual genetic contributions. A reanalysis of type-I diabetes data detects several susceptibility loci not previously found with other analysis methods.

**Linkage analysis of a dichotomous trait incorporating an associated quantitative trait: combining the likelihoods of IBD sharing and variance component methods.** *Y. Jiang*<sup>1</sup>, *J. Huang*<sup>2,3</sup>. 1) Preventive Medicine and Epidemiology, Loyola University Medical Center, Maywood, IL; 2) Department of Statistics and Actuarial Science, University of Iowa, Iowa City, IA; 3) Department of Biostatistics Division of Statistical Genetics, University of Iowa, Iowa City, IA.

Many complex disorders, such as asthma, autism, diabetes, and schizophrenia, are studied as dichotomous traits, but are also associated with certain quantitative biological markers or physiological risk factors. Incorporation of the correlational information between the dichotomous and the associated quantitative trait can increase the power to detect genes that predispose a disease trait. We proposed a likelihood based linkage analysis method that jointly consider the dichotomous and the quantitative trait for sib pair data. This likelihood is based on the conditional probability of the quantitative trait and all the marker data given the affection status, to account for the fact that the families collected for linkage analysis are usually ascertained based on affection status. We write this conditional probability as the combination of a likelihood based on IBD sharing scores given the dichotomous trait status and a variance component likelihood for the quantitative trait. A likelihood ratio (LR) test for linkage can be based on this likelihood. We conducted simulation studies to evaluate the properties of the proposed LR test, and found that it in general has higher power to detect linkage than the test based on IBD sharing but not incorporating the correlated quantitative trait.

**Evidence for Genetic Heterogeneity in Brachydactyly Type A1.** *E. McCready*<sup>1,2</sup>, *C.M. Armour*<sup>1,3</sup>, *A.G.W. Hunter*<sup>3</sup>, *D.E. Bulman*<sup>1,2</sup>. 1) Department of Microbiology and Immunology, University of Ottawa; 2) Ottawa Health Research Institute; 3) Eastern Ontario Regional Genetics Program, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

Brachydactyly Type A1 (BDA1) is a rare, congenital disorder that affects normal bone development, resulting in shortened digits. The phenotype of affected individuals can range from a severe form, in which the middle phalanges are absent or fused to the terminal phalanges, to a mild form, in which shortness of the middle phalange is more pronounced in digits 2 and 5 than the remaining digits. Recent genetic linkage studies have mapped the disease locus to chromosome 2q35-36 in two unrelated Chinese kindreds. Previous evidence of a balanced translocation between chromosome 5q and 17q in a young girl with BDA1 and Klippel-Feil anomaly further suggests that there may be a second BDA1 locus on either chromosome 5 or 17. To examine the location of genes that cause BDA1 we initiated a genome-wide scan in a four-generation, Canadian family with mild disease. Affected individuals in the family were characterized by shortened middle and distal phalanges, proximal first phalanx and fifth metacarpal. Affected individuals also tended to be of short stature. Polymorphic markers from across the genome, with particular emphasis on markers from chromosomes 5 and 17, were examined in 34 family members including 20 affected individuals. Using two-point linkage analysis, a maximum LOD score of 6.91 was observed at marker D5S477 (recombination fraction = 0.00), thus supporting linkage of BDA1 to this region. Haplotype analysis further defined an 11cM critical interval on chromosome 5p13.3-13.2. Preliminary scans of candidate genes from the region, including cadherin-6 and NPR3, have excluded mutations in the coding regions of these genes as the cause of the disease. This region represents a novel locus for BDA1 and provides evidence for genetic heterogeneity in this disease.



**Haplotype Data Mining for Optimization of Rheumatoid Arthritis Fine Genome Scan.** *J. Osorio y Fortea, S. Cailleau Moindrault, E. Petit, G. Bana, C. Pierlot, V. Arbelaz, P. Dieude, T. Bardin, B. Prum, F. Cornelis.*

GenHotel/Laboratoire de Recherche Europeen pour la Polyarthrite Rhumatoide, Genopole 91057, Evry, France/ECRAF (European Consortium on Rheumatoid Arthritis Families).

**Background.** Rheumatoid arthritis (RA) is a multifactorial disease. To search for RA loci, a genome scan was performed in 88 RA sib-pair families with 1109 microsatellite markers, providing an average spacing of 3,5 cM. All families were not informative for every marker, leading to a lack of genetic information. The density of the scan together with the informativity of the markers provided an opportunity to rescue the missing information through haplotyping.

**Objective.** Use of haplotype data mining to optimize linkage analysis.

**Method.** Haplotyping of the 88 families was performed for the first 756 markers with Genehunter2 program. The genotype from 2 flanking informative markers was used to complete the missing data for non-informative markers when those markers were spaced by less than 10 cM, assuming that the probability of double crossing over was negligible (<1%). A new linkage analysis was then performed with Sibpairna4 program from the package Analyze. Suggested RA loci were defined by markers with linkage  $p < 0.05$ .

**Results.** Haplotyping led to an average of 20% increase of informative events (parents informative for the transmission of identical by descent alleles to affected sib-pair). Linkage analysis of the 756 markers, which had suggested 53 susceptibility loci, showed after haplotyping 65 new loci. Apparent double recombinants were detected, pinpointing probable genotyping errors.

**Conclusion.** Haplotype data mining of fine genome scan data contributes to linkage analysis optimization. In addition to the identification of new loci and error-detection, haplotyping helps classifying families according to IBD at a candidate locus, to search for interaction between susceptibility loci.

**Linkage of familial membranoproliferative glomerulonephritis, type III to chromosome 1q31-32.** *J.J. Neary<sup>1</sup>, F. Zhang<sup>2</sup>, B. Zhao<sup>2</sup>, M. Booze<sup>2</sup>, S. West<sup>2</sup>, P.J. Conlon<sup>1</sup>, J.M. Vance<sup>2</sup>, M.A. Pericak-Vance<sup>2</sup>, W.K. Scott<sup>2</sup>, M.P. Winn<sup>2</sup>.* 1) Department of Nephrology, Beaumont Hospital, Dublin 9, Ireland; 2) Department of Medicine, Duke University Medical Center, Durham, NC.

Membranoproliferative glomerulonephritis type III is a chronic, progressive renal disease. The diagnosis is based on renal pathology, specifically immunofluorescence and ultrastructural appearance. The renal disease is characterized by mesangial cell proliferation as well as subendothelial and subepithelial deposits. The clinical features of MPGN are usually chronic in nature and include the nephrotic syndrome (high grade proteinuria, hypoalbuminemia, edema, hyperlipidemia, lipiduria, and hypercoagulability and frequently hypertension) and hematuria. Renal dysfunction occurs in ~50% of patients. Progression to end-stage renal disease (ESRD) is variable, but some patients stabilize and even improve. Here we present an Irish family in which there are nine affected members over three generations and the condition is inherited in an apparent autosomal dominant fashion. This is the only reported family with an inherited form of MPGN, type III. A stringent classification system was adopted to increase the power of the analysis. Affected subjects were defined as those with renal biopsy confirmation or who had end-stage renal disease or persistent and significant proteinuria or haematuria. A genome-wide scan was performed in the Duke Center for Human Genetics using a panel of 405 polymorphic microsatellite markers that define a 10cM resolution index map. Four separate markers on chromosome 1q31-32 generated strong evidence (two-point lod scores > 2) for linkage; one marker generated a peak LOD score of 3.24 at Q=0.00. Further fine-mapping studies are currently underway. This is the first time that MPGN, type III has been mapped to a locus and several plausible candidate genes are located in the region.

**Jouberts Syndrome: extension of genetic linkage to chromosome 9q34.3.** *L.C. Keeler<sup>1,2</sup>, E.P. Leeflang<sup>1</sup>, L. Sztriha<sup>3</sup>, L. Al-Gazali<sup>3</sup>, M. Nour-E-Kamal<sup>3</sup>, P.M. Frossard<sup>4</sup>, R. Bayoumi<sup>5</sup>, K. Saar<sup>6</sup>, F. Rueschendorf<sup>6</sup>, M. Reis<sup>6,7</sup>, B. Ben-Zeev<sup>8</sup>, J.G. Gleeson<sup>1,2</sup>.* 1) Neurosciences Graduate Program, U. Calif., San Diego, La Jolla, CA; 2) Neurosciences, U. Calif., San Diego, La Jolla, CA; 3) Paediatrics, United Emirates U., Al Ain, United Arab Emirates; 4) Pathology, United Emirates U., Al Ain, United Arab Emirates; 5) Biochemistry, United Emirates U., Al Ain, United Arab Emirates; 6) Medicine, Sultan Qaboos U., Al-Khod, Muscat, Sultanate of Oman; 7) Mikrosatellitenzentrum, Max-Delbrück-Centrum; 8) Institute of Human Genetics, Charité, Humboldt University, Berlin; 8) Sheba Meed. Ctr., Ramat-Gan, Israel.

Jouberts syndrome is a rare autosomal recessive disorder characterized by complete absence or significant hypoplasia of the cerebellar vermis. Common clinical features include episodic tachypnea and apnea in the neonatal period, abnormal eye movements, hypotonia, and developmental delay. Pathological examination suggests possible defects in hindbrain patterning, neuronal migration, and axon guidance. Jouberts is phenotypically heterogeneous; clinical features vary even between identical twins, suggesting partial penetrance for some phenotypes. Recently, homozygosity mapping revealed linkage to a 13-cM region of chromosome 9q34.3 in 2 consanguineous Omani pedigrees with a peak two-point LOD score of 3.7 at  $\theta = 0$  (Saar et al., 1999). We have ascertained two additional multiplex pedigrees, one consanguineous and one non-consanguineous, that suggest linkage to this interval. We are currently performing candidate gene analysis of 17 known genes and several ESTs in this region from all four 9q34.3 linked pedigrees. One Palestinian and one Iranian family, with distinct clinical phenotypes, did not show linkage to this region, suggesting genetic heterogeneity. We have obtained DNA samples from a large Pakistani pedigree that also does not link to this region. We are currently performing whole genome mapping in this family.

**A new locus for autosomal dominant high myopia maps to chromosome 17q21-23.** *T. Young*<sup>1,2</sup>, *P. Paluru*<sup>1</sup>, *E. Heon*<sup>3</sup>, *K. Bebhuck*<sup>2</sup>, *C. Armstrong*<sup>2</sup>, *S. Ronan*<sup>2</sup>, *A. Holleschau*<sup>2</sup>, *J. Petersen*<sup>2</sup>, *A. Alvear*<sup>2</sup>, *S. Wildenberg*<sup>2</sup>, *R. King*<sup>2</sup>. 1) Children's Hospital of PA, Philadelphia, PA; 2) University of Minnesota, Minneapolis MN; 3) Hospital for Sick Children, Toronto Canada.

**Purpose:** To map the gene(s) associated with autosomal dominant high myopia. **Methods:** A multi-generation French-Canadian family with autosomal dominant (AD) severe myopia was ascertained. Myopic individuals had no clinical evidence of syndromic disease, anterior segment abnormalities, or glaucoma. The family contained 87 individuals and DNA was available for 31 (10 affected). The average age of diagnosis of myopia was 8.9 years (range 2-11). The average spherical equivalent refractive error for affected adults was -13.925 diopters (range -5.50 to -50.00). The most severely affected individual had axial length measurements of 35.85 and 35.64mm OD and OS, respectively. Two-point linkage analysis using the FASTLINK program was run at 90% penetrance, using a myopia gene frequency of 0.0133. Linkage was excluded for microsatellite markers specific to two previously identified AD high myopia loci on chromosomes 18p11.31 and 12q22-q23. Syndromic myopia linkage was excluded using intragenic or flanking markers for Stickler syndromes type 1, type 2 and type 2B, Marfan syndrome, Ehlers Danlos syndrome type 4, and juvenile glaucoma prior to a full genome screen. **Results:** A two-point LOD score of 2.53 was obtained with microsatellite marker D17S949. A LOD score of 4.05 for marker D17S1290 was obtained after fine-point mapping using 35 additional flanking markers. Haplotyping defined an interval of 11 cM at chromosome 17q21-23. **Conclusion:** A third locus for autosomal dominant high myopia has been confirmed. Gene identification for this common complex disorder should provide insight into its pathophysiology. Supported by Research to Prevent Blindness, Inc, and the National Eye Institute, NIH.

**Linkage Disequilibrium Mapping of a Psoriasis Susceptibility Locus at 3q21.** *C.G. See<sup>1</sup>, D.R. Hewett<sup>1</sup>, J. Polding<sup>1</sup>, K. Cantone<sup>1</sup>, D. Smart<sup>1</sup>, J.H. Riley<sup>1</sup>, L. Samuelsson<sup>2</sup>, F. Enlund<sup>2</sup>, T. Martinsson<sup>2</sup>, J. Wahlstrom<sup>2</sup>, G. Swanbeck<sup>3</sup>, I.J. Purvis<sup>1</sup>.* 1) Molecular Genetics, GlaxoSmithKline, Stevenage, Hertfordshire, England; 2) Department of Clinical Genetics, Gothenberg University Hospital/East, S-416.85 Gothenberg, Sweden; 3) Department of Dermatology, Gothenberg University, Sahlgrenska University Hospital, S-413 Gothenberg, Sweden.

A genome wide linkage analysis has identified a psoriasis susceptibility locus at 3q21 in patients from southwestern Sweden. Preliminary linkage disequilibrium mapping with CA repeats narrowed the size of the candidate region to an estimated 1 Mb, an interval bounded by a single YAC. A set of 20 novel single nucleotide polymorphisms (SNPs) were identified from this region. The SNPs were ordered by mapping to a series of recombinant YACs with nested deletions. Genotyping of 188 families for these novel SNPs was performed by a combination of PCR-RFLP and Taqman technologies. Linkage disequilibrium mapping using TDT analysis showed 3 markers with significant disease association (p value < 0.05). The 150kb spanning these 3 SNPs has been sequenced. Additional markers and candidate transcripts are currently being isolated.

**Linkage and interaction of loci on 1q23 and *IBD1* may contribute to susceptibility to systemic lupus**

**erythematosus (SLE).** *B.P. Tsao*<sup>1</sup>, *J.M. Grossman*<sup>1</sup>, *S.K. Kim*<sup>1</sup>, *D.J. Wallace*<sup>2</sup>, *C-J. Chen*<sup>3</sup>, *C.S. Lau*<sup>4</sup>, *E.M. Ginzler*<sup>5</sup>, *R. Goldstein*<sup>6</sup>, *N. Shen*<sup>7</sup>, *K.C. Kalunian*<sup>1</sup>, *F.C. Arnett*<sup>8</sup>, *B.H. Hahn*<sup>1</sup>, *R.M. Cantor*<sup>9</sup>. 1) Dept Medicine, Div Rheumatol, UCLA, Los Angeles, CA; 2) Cedars Sinai Research Institute, Los Angeles, CA; 3) Kaohsiung Medical U, Kaohsiung, Taiwan; 4) Hong Kong U, Hong Kong, China; 5) SUNY, Brooklyn, NY; 6) U Ottawa, Ontario, Canada; 7) Shanghai Second Medical U, China; 8) U Texas-Houston, Houston, TX; 9) Dept. Human Genetics, UCLA, Los Angeles, CA.

SLE is an autoimmune disease caused by deposition of self-reactive autoantibodies and immune complexes resulting in inflammation and tissue injury. Six genome scans of different SLE multiplex family cohorts support multiple putative susceptibility loci. Seeking replication in an independent sample, we examined candidate loci (1q23, 14q21-23, 16p12-q13 [*IBD1*; inflammatory bowel disease 1], and 20p12) using a cohort of 109 multiethnic nuclear families containing 135 SLE-affected sibpairs. Model-free, multipoint linkage analyses (SIBPAL2, S.A.G.E. 4.0) showed evidence for linkage at 1q23 (peak at D1S484, mean allele sharing [MAS] of .57,  $p = .0008$  by variance component regression analysis of the combined data from the 135 affected sibpairs, 205 SLE discordant sibpairs, and 72 unaffected sibpairs) and at *IBD1* (peak at D16S3136, MAS = .59,  $p = .02$ ). Linkage evidence at 20p12 was weak (MAS = .52 to .55,  $p = .04$  to .07). Linkage to 14q21-23 was excluded ( $I_s = 1.8$ ) by a multipoint linkage analysis. Because *IBD1* has been mapped in genome scans of several autoimmune diseases, we postulated that it might harbor an autoimmune-modifier gene. Thus, we examined the potential interaction between *IBD1* and 1q23, and between *IBD1* and 20p12. The distribution of haplotype sharing at 1q23 increases as the degree of haplotype sharing at *IBD1* also increases ( $p = .0055$  by the Jonckheere-Terpstra exact test). No evidence of an interaction was observed between *IBD1* and 20p12. Analysis of pedigrees conditioned on those showing linkage to *IBD1* increases allele sharing at 1q23 (MAS = .66). Exploiting interaction between linked region may facilitate gene mapping efforts.

**Genome Scans for Apolipoproteins A-I and B in the NHLBI Family Heart Study (FHS).** *K.A. Weis<sup>1</sup>, D.K. Arnett<sup>1</sup>, M.Y. Tsai<sup>2</sup>, H. Coon<sup>3</sup>, G. Heiss<sup>4</sup>, L.D. Atwood<sup>5</sup>, Y. Hong<sup>6</sup>.* 1) University of Minnesota, Minneapolis, MN; 2) Fairview-University Medical Center, Minneapolis, MN; 3) University of Utah, Salt Lake City, UT; 4) University of North Carolina, Chapel Hill, NC; 5) Boston University School of Medicine, Boston, MA; 6) Washington University, St. Louis, MO.

Low plasma levels of apolipoprotein A-I, the major protein component of HDL, and elevated plasma levels of apolipoprotein B, the major protein constituent of LDL, are associated with increased risk of coronary heart disease. Several studies have shown that plasma apo A-I and B levels are influenced by genetic factors. Recent genome-wide scans have shown linkage to previously uncharacterized genomic regions, indicating additional loci influencing apolipoprotein levels. We conducted genome-wide scans for apo A-I and apo B using two subgroups from the NHLBI Family Heart Study (FHS): 1) sibships with at least one sibling at increased risk for CHD (937 white individuals in 355 families genotyped by the University of Utah for 243 markers) and 2) 401 families selected as the largest pedigrees in FHS with the greatest familial variation in CHD risk (1048 white individuals in 305 families genotyped by the Mammalian Genotyping Service (MGS) for 402 markers). A genome-wide scan was also performed on a non-redundant combination of these two subgroups (1321 white individuals in 473 families with 645 anonymous markers). Multipoint variance components linkage analysis was conducted using GENEHUNTER2. Apo A-I and B were adjusted for field center in a sex-specific linear regression model, and standardized residuals were calculated. These residual phenotypes were further adjusted in GENEHUNTER2 with the direct addition of standardized age, age<sup>2</sup>, and body mass index. For apo A-I, a maximum LODscore of 1.88 was obtained with the combined marker set on chromosome 12 at 83.2 cM, near marker D12S1052. For apo B, a maximum LODscore of 1.53 was obtained with the MGS marker set on chromosome 10 at 93.9 cM (LODscore decreased to 1.44 in the combined analysis), near marker D10S1432. In conclusion, our study did not find any chromosomal regions significantly linked to apolipoproteins A-I or B in the FHS population.

**Genome scan on fifty-six multiplex bipolar pedigrees collected by the NIMH Genetics Initiative (Bipolar Disorder).**

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The NIMH Genetics Initiative (Bipolar Disorder) has ascertained 153 multiplex bipolar pedigrees that have been genotyped in two waves. The results of the wave one analyses have been reported (Nurnberger et al., 1997). We are now reporting the wave two genome scan results for the twelve chromosomes that Johns Hopkins has genotyped: chromosomes 2, 4, 7, 9, 11, 13, 14, 18, 19, 20, 21, and X. The microsatellite marker maps range from 5.8 cM (chromosome X) to 10.3 cM (chromosome 7). Three affection status models (ASM I-III) were used in the linkage analyses. Multipoint nonparametric analyses using GENEHUNTER v 2.0 and XGENEHUNTER v 1.3 produced three candidate regions. A broad peak that spans 4q32-4q35 is visible under all affection status models; it reaches its maximum NPL of 2.7 ( $p=0.005$ ) under ASMI. The broad peak at 20p12 reaches an NPL of 2.63 ( $p=0.006$ ) under ASMI. The 11p15 peak reaches an NPL 2.42 ( $p=0.006$ ) under ASMI. All three of these regions have been previously implicated in bipolar disorder. Continued high-density genetic mapping in these regions is warranted.



**Genetic founder effect at the hereditary neuralgic amyotrophy (HNA) locus on chromosome 17q25.** *G.D.J. Watts, K.C. O'Briant, P.F. Chance.* Pediatrics, University of Washington, Seattle, WA.

Hereditary neuralgic amyotrophy (HNA) is an autosomal dominant disorder that is associated with episodic, recurrent brachial plexus neuropathy. To date no families other than those from Caucasian decent have been reported. Recent studies mapped the HNA locus to chromosome 17q25. To further refine the HNA locus we carried out genetic linkage studies in four pedigrees with a high density set of DNA markers from chromosome 17q25. All pedigrees demonstrated linkage to chromosome 17q25 and an analysis of recombinant events placed the HNA locus within an approximate 1Mb interval flanked by markers D17S722 and D17S802. In order to test the power of linkage disequilibrium mapping we compared genotypes of 12 markers from probands of 7 pedigrees of European extraction, that show linkage to chromosome 17q25. This identified a founder effect in 6 of the 7 pedigrees with a minimal shared haplotype that further refines the HNA locus to an approximate 500Kb interval. These findings suggest that for the pedigrees from the United States at least one mutation in the HNA gene was introduced from European roots.

**Evidence of linkage for keratoconus to chromosome 16 based on a genome-wide linkage analysis.** *H. Tynnismaa*<sup>1,2</sup>, *P. Sistonen*<sup>3</sup>, *S. Tuupanen*<sup>1,2</sup>, *T. Tervo*<sup>4</sup>, *A. Dammert*<sup>5</sup>, *T. Latvala*<sup>5</sup>, *T. Alitalo*<sup>1,2</sup>. 1) Dept Obstetrics and Gynecology, Helsinki University Central Hospital, Finland; 2) Dept Medical Genetics, University of Helsinki, Finland; 3) Finnish Red Cross, Blood Transfusion Service, Helsinki, Finland; 4) Dept of Ophthalmology, Helsinki University Central Hospital, Finland; 5) Dept of Ophthalmology, University of Oulu, Finland.

Keratoconus (MIM148300) is a noninflammatory corneal thinning and irregular steepening disorder with an incidence of 1:2000. In western populations the disease is the leading single indication for corneal transplantation surgery. Most of the cases are sporadic. It has been suggested that 6-10% of the keratoconus cases follow autosomal dominant inheritance with incomplete penetrance or variable expression. The pathogenesis of the disorder is unclear. Due to scarcity of large family material, no genome-wide linkage scans to localize the gene/genes have been published. Here we present the first results of genome-wide linkage analysis, which was performed using DNA samples of 20 keratoconus families from Northern Finland. The diagnosis of the patients was based on computer-assisted videokeratoscopy. Linkage analysis was performed using the GENEHUNTER program, assuming autosomal dominant inheritance and a gene penetrance of 0.5. The initial screen was performed using a panel of 369 markers with an average spacing of 10cM. This resulted in four promising regions on chromosomes 2,10,15 and 16, with LOD scores >1.5. Additional flanking markers were genotyped and increased multipoint LOD scores were obtained only for the markers on chromosome 16. Strong evidence for linkage was observed on chromosome 16 with a maximum multipoint LOD score of 4.10 and non-parametric linkage (NPL) scores of 3.27 ( $p=0.00006$ ) for the same locus. We are currently fine-mapping the candidate region on chromosome 16 as a first step to isolate the keratoconus gene. Even though it is not yet known whether the gene on chromosome 16 will be defective in keratoconus patients world widely, identifying the keratoconus gene in Finnish families will improve the understanding of the pathogenesis of this complex trait.

**Studies on Charcot-Marie-Tooth disease with vocal fold paralysis (CMT2C) reveal further evidence for genetic heterogeneity.** *I. Puls<sup>1</sup>, K. Bidus<sup>2</sup>, D. Drayna<sup>5</sup>, M.K. Floeter<sup>4</sup>, E. Mann<sup>2</sup>, J.P. Taylor<sup>1</sup>, K.R. Wagner<sup>3</sup>, K.H. Fischbeck<sup>1</sup>, C. Ludlow<sup>2</sup>.* 1) Neurogenetics Branch (NINDS), NIH, Bethesda, MD; 2) Laryngeal and Speech Section (NINDS), NIH, Bethesda, MD; 3) Johns Hopkins University, Neurology Department, Baltimore, MD; 4) Electromyography Section (NINDS), NIH, Bethesda, MD; 5) Section on Transcription Factors, Sensory Receptors and Channels (NIDCD), NIH, Bethesda, MD.

CMT2C is a motor-sensory polyneuropathy with autosomal dominant inheritance and vocal fold paralysis that is caused by axonal degeneration of peripheral nerves. Two families with this particular phenotype have been linked to chromosomes 2q14 and 6q16. We collected 4 different families from the United States with no evidence of a common ancestor. The patients showed signs of distal weakness and atrophy with mild sensory loss and uni- or bilateral vocal fold paralysis. The onset of symptoms and disease progression were variable both within and between the families examined. Nerve conduction studies gave evidence for axonal polyneuropathy. Linkage to known loci for CMT2C was tested, using standard protocols and published polymorphic markers. The analysis suggested linkage to chromosome 2q14 for one family. Linkage to chromosome 2 and 6 could be excluded for the other three. Our results indicate that there is genetic heterogeneity for CMT with vocal fold paralysis, with at least a third locus existing in addition to the two previously described. Extension of our pedigrees and a genome wide screen is in progress.

**Linkage Analysis of Nonsyndromic Cleft Lip and Palate in 30 Filipino Families with Multiple Affected**

**Individuals.** *R. Schultz*<sup>1</sup>, *S. O'Brien*<sup>1</sup>, *S. Daack-Hirsch*<sup>1</sup>, *M. Cooper*<sup>2</sup>, *M. Marazita*<sup>2</sup>, *J. Murray*<sup>1</sup>. 1) Dept Pediatrics, University of Iowa, Iowa City, IA; 2) Cleft Palate Center, University of Pittsburgh, Pittsburgh PA.

Cleft lip and palate (CL/P) is a congenital anomaly present in on average 1/1000 live births. About 30% of cases are syndromic, with various causes such as Mendelian disorders, chromosomal anomalies, or teratogen exposure. The remaining 70% are nonsyndromic (NS), in which the affected individual has no other abnormalities. Several loci were selected for study to determine whether they demonstrated linkage to NS CL/P. Loci were chosen based on previous suggestive findings and include 1p36, 4p16.2 (MSX1), 2p13.2 (TGFA), 14q24.3 (TGFB3), 4q31, 6p23, and 19q13. Microsatellite repeat or other highly polymorphic markers in these regions were selected. 30 Filipino families comprising 292 individuals of whom 114 are affected were used for the linkage study. An additional 63 families comprising 149 affected individuals are available for replication of any significant or suggestive results obtained from the initial 30 families. The MSX1-CA repeat and the microsatellite repeat marker D6S1029 have been genotyped for all 30 families. Parametric linkage analysis was carried out by the LINKAGE program with the FASTLINK update, and nonparametric analysis was carried out by GENEHUNTERnpl. The Family Based Association Test 1.2 program was used to perform the TDT analysis. The MSX1 repeat gave a maximum LOD score of 1.20 at theta=0.2, a SIMIBD p-value of 0.2, and a multiple allele p-value for the TDT of 0.34. D6S1029 had a maximum LOD score of 0.31 at theta=0.3, a SIMIBD p-value of 0.06, and a multiple allele p-value for the TDT of 0.76. The LOD score of 1.26 for the MSX1-CA repeat and the SIMIBD p-value of 0.06 for D6S1029 are suggestive of linkage, although not significant. Genotyping is being extended to other markers in these regions and to the additional 63 families for validation. Since the mouse knockout of *Msx1* has cleft palate and MSX1 mutations have been found in rare cases of apparent NS CL/P, this locus is very plausible for linkage.

**LINKAGE MAPPING TO CHROMOSOME 3q24 AND EVIDENCE FOR A FOUNDER EFFECT FOR HERMANSKY-PUDLAK SYNDROME IN CENTRAL PUERTO RICO.** *J.R. Toro, W.A. Gahl, S. Bale.* National Institutes of Health, NIH, Bethesda, MD.

Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding diathesis due to platelet storage pool deficiency, and lysosomal accumulation of ceroid lipofuscin. HPS occurs with a high frequency in northwest Puerto Rico (PR), where 1 in 21 individuals is a carrier. HPS patients from northwest PR are homozygous for a 16-bp duplication in exon 15 of the HPS1 gene; this mutation is associated with significant risk of pulmonary fibrosis. The objective of this study is to determine the phenotype and genotype of HPS patients from central PR and map the gene for a new subtype of HPS. All patients lacked platelet dense bodies on electron microscopy, confirming the diagnosis of HPS. All patients also lacked the 16-bp duplication in HPS1. All patients had ancestry from central PR and exhibited only mild visual defects and mild pigment dilution of hair, eyes and skin. These findings differed significantly from HPS individuals with the 16-bp duplication in HPS1. Using homozygosity mapping on pooled DNA from 6 families from central PR, we mapped the putative new HPS susceptibility gene to chromosome 3q24. This region on chromosome 3q24 was analyzed using 21 polymorphic markers. Contiguous homozygous markers defined an ancestral haplotype in all affected individuals. Recombination allowed us to tentatively narrow the critical region to a 1.6 cM interval on chromosome 3q24. The ancestral haplotype encompassed at least 11 cM and we estimated that the recombination with the ancestral chromosome has occurred in approximately 44% of cases. Using the Luria & Delbruck method, we estimated that the mutation occurred in central Puerto Rico approximately in 1880-1890. History books, church records and genealogical studies revealed that common ancestors of three of our families emigrated from the town of Ciales to the towns of Aibonito and Barranquitas because of harsh economic conditions in Ciales approximately. It is possible that genetic isolation of the mountainous terrain of this area contributed the development of this second founder mutation causing HPS in central Puerto Rico.

**Loci on Chromosomes 7 and 2 Interact to Increase Linkage Evidence in Autistic Disorder.** *Y. Shao<sup>1</sup>, W.K. Scott<sup>1</sup>, K.L. Raiford<sup>1</sup>, C.M. Wolpert<sup>1</sup>, A. Ashley-Kock<sup>1</sup>, M.L. Cuccaro<sup>2</sup>, J.R. Gilbert<sup>1</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Ctr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Our genomic screen analysis of 99 autistic disorder (AutD) families revealed suggestive evidence for linkage to chromosome 2q at D2S116 (198 cM; MLS=1.12). In addition analysis of linkage disequilibrium for D2S116 showed an allele-specific p-value < 0.01. Recently, Buxbaum et al. (2001) also reported linkage to the same 2q region (Heterogeneity LOD score (HLOD) = 1.96) in an independent genome screen. Their evidence for linkage increased (HLOD=2.99) when they restricted their analysis to the subset of AutD patients with delayed onset (>36 months) of phrase speech (PSD). We similarly classified our AutD sibships, identifying 37 AutD families with PSD. Analysis of our PSD subset increased support for to 2q linkage (MLS =2.82,HLOD=2.05. The evidence for heterogeneity decreased in the PSD subset (the proportion of linked families (a) increased from 0.25 to 0.90). We observed similar linkage enhancement in the PSD subset at another candidate region on Chr7. Our MLS score increased from 1.17 to 2.00 and the HLOD from 0.67 to 1.46. The evidence for heterogeneity also decreased (a increased from 0.30 to 0.90). We also observed evidence for statistical interaction between these 2 unlinked regions in the overall dataset. The non-parametric lod score (LOD\*) from GENEHUNTER+ for chr7=1.09 in baseline analysis but increased to 2.97 when families were weighted by their evidence for linkage at d2s116. The conservative c-square test with df =1 yielded a p-value <0.005 to show the significance of the interaction with the increased weighted lod. Significant interaction between unlinked regions as well as phenotypic homogeneity provides additional evidence that loci from these two regions contribute to AutD risk and suggests that this is a powerful approach to identify susceptibility genes for AutD.

**Haplotype analysis in patients with velopharyngeal insufficiency (VPI) minimizes one VPI locus in 2.8Mb region of 22q11.** *L.P. Tsai<sup>1</sup>, K.S. Tsou<sup>2</sup>, Y.R. Shi<sup>3</sup>, J.Y. Wu<sup>3</sup>, F.J. Tsai<sup>3</sup>*. 1) Dept Pediatrics, Taipei Mun Womens/Child Hosp, Taipei; 2) Dept Pediatric Psychiatry, Taipei Mun Womens/Child Hosp, Taipei; 3) Dept Medical Research, China Medical College Hospital, Taichung, Taiwan.

Velocardiofacial syndrome (VCFS) and DiGeorge syndrome (DGS) exhibit a wide spectrum of anomalies, including cleft palate, congenital heart defects of the conotruncal type, facial dysmorphism, cellular immunity disorder and learning disabilities. Most patients were hemizygous in the region 22q11, and share a 3-Mb deletion flanked by D22S427 and D22S306/308. Most of the remaining cases had a smaller, 1.5-Mb deletion. However, there was no correlation between the phenotype and the presence or size of the deletion so far. To define the specific deletion that is responsible for certain phenotype, instead of study on complex VCFS/DCS patients, we used the eleven consecutive simple tandem repeat polymorphic markers mapped to 22q11 to genotype patients of isolated phenotype. Haplotype analysis was performed with patients and their parents. In this way, we found that five patients of velopharyngeal insufficiency (VPI) free from congenital heart defects had a similar 2.8-Mb deletion flanked by D22S1623 and D22S303, which is distal to the most common 3-Mb deletion region of VCFS/DGS with a 1.7-Mb overlap. Most patients with conotruncal heart defect had deletion more proximal to this 2.8-Mb region. Our data suggest that one gene or a set of genes within this 2.8Mb region is essential for velopharyngeal function. Haploinsufficiency of these genes cause symptoms of VPI clinically. Compared with the previous reports, it seems that molecular analysis on isolated phenotype patients related to VCFS is a better approach to get phenotype/genotype correlation. We recommend that haplotype analysis is needed for patients with VPI to further narrow down the region and look for the candidate gene(s) for VPI.

**Genetic Analysis of Kindler Syndrome.** *D.H. Siegel<sup>1</sup>, H. Penagos<sup>2</sup>, C.B. Wiebe<sup>3</sup>, H.S. Feiler<sup>4</sup>, K.C. Wilhelmsen<sup>4</sup>, E.H. Epstein<sup>1</sup>.* 1) Dermatology, UCSF, San Francisco, CA; 2) Dermatology, Chiriqui Regional Hospital, Social Security Bureau of Panama, David, Panama; 3) Oral Biological and Medical Sciences, Faculty of Dentistry, University of British Columbia, Vancouver, BC, Canada; 4) Ernest Gallo Clinic and Research Center, Department of Neurology, UCSF, Emeryville, CA.

Kindler syndrome is a rare autosomal recessive disorder with changes resembling both epidermolysis bullosa and congenital poikiloderma. Twenty-six patients with Kindler syndrome from the Ngobe tribe of Bocas del Toro, Panama were examined, and blood samples were collected. Abnormalities included congenital acral blisters, photosensitivity, poikiloderma, cigarette paper-like wrinkling of the dorsal hands and feet, periodontal disease, phimosis in males, and webbing of the digits. Several regions of decreased allelic heterogeneity were identified in an 811 marker genome-wide scan on pooled DNA samples. A subsequent genome-wide scan of DNA from 16 individual patients and 8 related unaffected controls confirmed one of these, with a maximum LOD score of 2.48. Individual genotyping defined a 300-Kb area of homozygosity in the Panamanian patients. Genotyping of DNA from additional patients from Japan, the Middle East, the United States and Canada confirmed this region of homozygosity containing four annotated genes. Mutational analysis is currently underway.



**The power of using classification and regression tree to reduce heterogeneity in the affected sibpair analysis.** *J.L. Chen<sup>1</sup>, C.J. Chang<sup>2</sup>, C.S.J. Fann<sup>1</sup>*. 1) Inst Biomed sci, Acad Sinica, Taipei, Taiwan; 2) Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan.

The power to detect linkage is always reduced if data consists of a mixture of heterogeneous sibpairs. To circumvent such problems, we proposed a method that uses cluster analysis, classification and regression tree with meaningful covariates to identify more homogeneous subgroups to detect genetic linkage for qualitative and quantitative traits previously. In this study, we examined the power of this method by considering a few factors, the extent of locus heterogeneity (percentage of unlinked family), sample size of sibpair and underlying assumption of related covariates using Monte Carlo simulation approach. Family data consist of sibpairs and ten covariates were generated according to some known distributions. Among these covariates, only four (three continuous and one binary) were related to assumed traits. An interaction between two covariates (one binary, one continuous) was also assumed. The algorithm implemented with classification and regression tree was able to pick up 4 related covariates about 70% of the time and it was about 90% to pick up 3 related covariates in 1000 replicates. The simulation results indicated the power was generally acceptable (>80%) regardless of any factor when sample size was large ( $n=500$ ). However, with small sample ( $n=100$ ), power dropped from 81% to 42% if ratio of covariate means increased from 0.5 to 0.8. Power was 74% and 52% when the percentage of unlinked family was 30% and 70% respectively. The presence of interaction reduced the power even further. According to our simulations, power was low in small sample if the percentage of unlinked family is more than 30% and ratio of covariate means is bigger than 0.65. In conclusion, this method performs well when sample size is large; however, caution should be taken with smaller sample size.

**Regressive Threshold Model for Sib Pair Analysis of Complex Diseases with Variable Age of Onset.** *L. Briollais.*  
Department of Epidemiology and Biostatistics, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, and  
Department of Public Health, University of Toronto, Toronto, Canada, M5G 1X5.

Most chronic human diseases exhibit variability in their age of onset and have a complex mode of inheritance. Model-free linkage analysis methods have become popular in gene mapping of complex diseases (CDs) but few of them can account for censored observations. We propose here a new regressive-based approach for sib-pair analysis of CDs with variable age of onset that allows for a residual familial correlation, measured risk factors and gene by environment interaction. The rationale is to assume that the disease is determined through an underlying continuous variable, e.g. the liability, normally distributed in the general population and correlated among family members. Affected individuals at age-of-onset  $k$  have a liability that lies between two time-dependent thresholds  $[T_k, T_{k+1}]$ , the thresholds demark the quantiles of the standard normal cdf. The first moment of the liability distribution is expressed as a function of measured risk factors and genotype risks at the marker locus. Conditionally to the proportion of alleles shared ibd, the covariance between sibs' liabilities is a sum of two products: the variance accounted for by the trait locus by the estimated proportion of allele shared ibd, and the variance resulting from a polygenic effect and/or shared environment by a residual sib-sib correlation. The likelihood is expressed using the truncated normal distribution theory and parameter estimation is performed by maximum likelihood methods. The null hypothesis of no linkage is tested by comparing the variance of the trait locus to 0. Our first simulation studies under different genetic models showed that the method was associated with acceptable levels of Type I error and a gain in power was obtained when a residual familial correlation was accounted for and in some cases in presence of gene by environment interaction. This approach can be easily extended to multivariate phenotypic traits, large families and multiple loci and thus provides a new opportunity for gene mapping of complex diseases.

**Construction of a Confidence Set of Markers for the Location of a Disease Gene Using Affected-Sib-Pair Data.** *S. Lin.* Dept of Statistics, Ohio State Univ, Columbus, OH.

We have previously proposed a confidence-set approach to find tightly linked genomic regions in parametric linkage analysis. Two fundamental advantages of this approach are (1) its ability to localize disease genes to small chromosomal regions at the stage of an initial genome-scan study and (2) the avoidance of the need of multiplicity adjustment for the number of markers tested. Here we extend the confidence-set approach to non-parametric linkage analysis of affected-sib-pair (ASP) data based on their identity-by-descend (IBD) information. Non-parametric methods are usually more suited for mapping complex traits since their underlying genetic models are often unclear. The confidence-set approach is based on testing a non-traditional formulation of hypotheses, with the null (tight linkage) and alternative (loose or no linkage) hypotheses being the "reverse" of the traditional formulation of null (no linkage) and alternative (linkage) hypotheses. From the corresponding theory between hypothesis testing and confidence set, construction of a confidence set of markers amounts to collecting the markers that are not rejected when testing the non-traditional formulation of hypotheses. Two well-known statistics in non-parametric linkage analysis are based upon for constructing confidence sets, the Two-IBD test (proportion of ASPs sharing two alleles IBD), and the Mean test (average number of alleles shared IBD in the APSs). In order to construct confidence sets using these two tests, we need to specify the marker IBD distribution, which is shown to be completely determined by either the disease incidence data or the variance components of a trait, without specifying a penetrance model. Our results show that the two main advantages of the confidence-set approach are retained when the method is generalized to non-parametric analysis. Furthermore, we study accuracy of confidence sets in terms of choice of tests, underlying disease incidence data, and amount of data available. From the results, we conclude, among other things, that the Mean test outperforms the two-IBD test in most situations, but the reverse is true for traits with small additive variance.

**Power of the ordered subset method for detection and localization of genes in linkage analysis of complex traits.**

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We have proposed the ordered subset method for identification and localization of genes for complex traits in genetically heterogeneous samples (Hauser et al. 1998; Ghosh et al. 2000). Leal and Ott (2000) have shown that stratification of such samples into genetically homogeneous subsets can provide increased evidence for linkage, depending on the strength of the genetic effect in the linked stratum and the size of the strata. However, in practice it may be difficult to identify strata prior to the linkage analysis. The ordered subset method does not require specification of individual strata prior to analyses but rather, uses trait-related covariates, such as mean age of onset, to rank families. The families are added to the analysis in rank order one by one and the maximum lod score is calculated as each family is added. The set of families providing the maximum subset lod score is identified as well as the maximum likelihood estimates of location and genetic effect for this subset. We test the null hypothesis that ordering families by the covariate does not improve the maximum subset lod score by calculating an empirical p-value. This p-value is estimated from the distribution of maximum subset lod scores obtained by randomly permuting the order of the families and recalculating the maximum subset lod score. We examine the power of this approach in detecting linkage in a simulation study using a variety of genetic heterogeneity models and relationships to a generic trait-related covariate. One appealing feature of this method is that the estimate of the location of the disease gene is recalculated in the subset. We examine improvements in disease gene localization using this ordered subsets method.

**A score test for detecting linkage to quantitative traits.** *H. Putter*<sup>1</sup>, *L.A. Sandkuijl*<sup>1,2</sup>, *J.C. van Houwelingen*<sup>1</sup>. 1) Dept of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands; 2) Dept of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

The two most popular methods to detect linkage of a quantitative trait to a marker are the Haseman-Elston (HE) regression method and the variance components likelihood ratio test. In the literature these methods are frequently compared and the relative advantages and disadvantages of each method are well known. In this paper, we derive a score test for the variance component attributable to a specific quantitative trait locus in a variance components model and show that for sib-pairs it is mathematically equivalent to a recently proposed version of the HE method that optimally combines the sum squared and the difference squared of the centered phenotype values of the sibs. Since score tests and likelihood ratio tests are equivalent for large sample sizes, the variance components likelihood ratio test is also asymptotically equivalent to this optimal HE test. This fact gives a theoretical explanation of the empirical observation from simulation studies reporting similar power of the variance components likelihood ratio test and the optimal HE method. Perhaps more importantly for practical purposes, the score test can also be extended in a natural way to support the simultaneous analysis of more than two subjects and multivariate phenotypes.

**Non-positive semidefinite identity-by-descent matrices for highly heritable traits lead to singularities in the extended pedigree variance components model.** *J.S.C. Ronald<sup>1</sup>, N. Ertekin-Taner<sup>2</sup>, S.G. Younkin<sup>1</sup>, L.H. Younkin<sup>1</sup>, M. Hutton<sup>1</sup>, S.G. Younkin<sup>1</sup>.* 1) Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Genetic Epidemiology, Mayo Clinic, Rochester, MN.

Quantitative traits have been used to map disease loci for thrombosis, obesity, and diabetes. We have successfully applied a similar approach to late-onset Alzheimers disease by performing linkage analysis on plasma amyloid b protein (Ab42) levels in extended pedigrees using the SOLAR variance components method. This method uses maximum likelihood to model the phenotype covariance matrix by decomposing it into locus specific, residual genetic, and environmental components. An essential requisite for accurate calculation of lod scores is that the fitted phenotype covariance matrix be non-singular. Provided that the locus specific identity-by-descent (ibd) matrix is positive semidefinite (psd), any nontrivial linear combination of the ibd, kinship, and identity matrices, each weighted by its appropriate variance component, will result in a positive definite matrix. While the true ibd matrix is always psd, an estimated ibd matrix may have negative eigenvalues. A non-psd ibd matrix ensures that there will be combinations of variance components in the interior of the parameter space that result in a singular phenotype covariance matrix. In this situation, likelihood maximization algorithms may produce arbitrarily high or low lod scores that are artifact of singularities. We have observed that the regression based method of Fulker et al. for estimating multipoint ibd (mibd) coefficients as implemented in SOLAR results in non-psd mibd matrices. In our experience, the smallest eigenvalue of typical mibd matrices is only slightly negative (on the order of  $-10^{-1}$ ), and therefore singularities only occur in models where the trait heritability is substantial ( $>85\%$ ). For a trait with heritability exceeding 85%, as occurs for plasma Ab42 in certain sets of families, several options are available. These include restricted searches of the parameter space and transformation of the mibd matrices, either of which ensures that the phenotype covariance matrix stays positive definite.

**A multivariate approach for detecting linkage with sib-pair observations.** *X. XU, L. Tian, X. Xu, L.J. Wei.* Program for Population Genetics, and Department of Biostatistics, Harvard School of Public Health, Boston, MA.

Under the circumstance that a single QTL contributes to multiple distinct phenotypic traits, a multivariate linkage test that analyzes multiple traits simultaneously would provide substantial power improvement over individual single-trait tests. We have previously described a simple test procedure extending the unified Haseman-Elston method to allow linkage test with multivariate traits (Xu et al, *Genet. Epidemiol.* 2001, in press). The test is based on linear combination of estimators of the H-E regression coefficient for each individual trait, and works optimally when the narrow-sense heritabilities of the QTLs for all traits are approximately equal. It is common that the true values of the narrow-sense heritabilities vary among traits and are unknown prior to analysis, reducing the efficiency of the multi-trait test. Here, we propose a modification to our previous multi-trait test by adding an empirically determined weight to estimators in the linear combination according to a function of the standardized estimators for the trait, with larger weights going to the traits with larger standardized estimators. We expect the modified method to perform better than the original multi-trait test under condition of unequal narrow-sense QTL heritabilities among different traits. The statistical basis of the modified methods will be discussed, and its type-I error and power will be compared with the original test using both simulated data and real observations. This statistical procedure can be generalized to other multivariate test such as association analysis.

**Suggestive linkage on chromosomes 1 and 15 for a protective alcohol-related phenotype.** *D.M. Dick<sup>1</sup>, J.I. Nurnberger, Jr.<sup>1</sup>, H.J. Edenberg<sup>1</sup>, A. Goate<sup>2</sup>, R. Crowe<sup>3</sup>, J. Rice<sup>2</sup>, V. Hesselbrock<sup>4</sup>, T. Foroud<sup>1</sup>.* 1) Indiana University, Indianapolis, IN 46202-5251; 2) Washington University, St. Louis, MO 63110; 3) University of Iowa, Iowa City, IA 52242-1000; 4) University of Connecticut, Farmington, CT 06030-2103.

Efforts to identify genes involved in alcohol use/abuse have been complicated by uncertainty regarding how best to define phenotypes for genetic analyses. Using data from the Collaborative Study on the Genetics of Alcoholism (COGA), we have been developing quantitative phenotypes characterizing patterns of alcohol use. COGA is a multi-site family-based study that has systematically ascertained alcoholic probands from treatment facilities. A total of 1,227 proband families and 234 control families were recruited in the initial stage of the study. Subjects completed a multi-diagnostic interview and personality questionnaire. A principal component analysis was performed on variables measuring aspects of alcohol use, antisociality, anxiety and guilt about one's drinking, and personality. Three components emerged: the first was characterized by drinking, drinking problems, and high novelty-seeking. The other two were protective: factor two was characterized by high harm avoidance and a later age of onset of drinking, and the third factor was characterized by high reward dependence. A genome screen was completed in 987 individuals from an initial sample of 105 families having at least three first-degree relatives diagnosed as alcohol dependent; a follow-up genomic screen was conducted on 1295 individuals from another 157 multiplex families. The three factors from the principal component analysis were used in a series of quantitative trait sibling pair linkage analyses. Chromosome 1 yielded consistent evidence of linkage to the protective factor two across the initial, replication, and combined samples, with a maximum lod score of 3.3 in the combined sample near the marker D1S518. Chromosome 15 also produced consistent evidence of linkage across the samples, with a maximum lod score of 2.0 between the markers D15S143 and GATA153 in the combined sample; this region is also linked to EEG findings in the COGA sample.



**Assessment of significance for quantitative traits via a permutation-type test.** *M. Abney<sup>1</sup>, C. Ober<sup>1</sup>, M.S.*

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Assessing significance, in particular, genome-wide significance, has proven to be a difficult problem in linkage mapping. One common solution has been to use a proposed "universal" cut-off value for the lod score. This solution, however, cannot provide a true p-value for any one particular study, as every study differs in design and in population considered. One might prefer to take these into account when assessing genome-wide significance. We propose a novel permutation-type method to assess genome-wide and locus-specific significance for tests for detection of linkage or linkage disequilibrium with quantitative traits. The method is applicable under a linear model for the phenotype, with covariates and/or correlated errors allowed. The method can be applied to studies composed of independent sampling units (e.g., independent sib-pairs) or large families with an arbitrarily complex (but known) correlation structure. The difficulty with permutation tests in the latter case arises from the lack of exchangeability. In the case of multivariate normality, we overcome this problem by finding a linear transformation of the phenotypic residuals (i.e., after accounting for age, sex and other covariates) such that the transformed residuals are uncorrelated. Permutations are then done on these uncorrelated residuals before untransforming them and obtaining simulated data sets. This method has the advantage of being an exact permutation test when the phenotype is distributed according to a multivariate normal distribution, and it can be robust to deviations from this assumption. We compare the method with other means of assessing significance and explore its robustness, which depends on the choice of linear transformation. For obtaining genome-wide p-values, our proposed method is applicable when positions of markers are independent of the observed linkage signal, under the null hypothesis (e.g., genome screen data), while there is no such restriction for locus-specific p-values. This work was supported by NIH grants DK55889, HG01645 and the NSF GIG postdoctoral fellowship.

**Genome-wide scan in Finnish families provides evidence for a locus for bipolar disorder on 4q28.3.** *J. Ekholm*<sup>1,3</sup>, *T. Kieseppä*<sup>2</sup>, *T. Partonen*<sup>2</sup>, *T. Paunio*<sup>1</sup>, *M. Perola*<sup>1,3</sup>, *J. Lönnqvist*<sup>2</sup>, *L. Peltonen*<sup>1,3</sup>. 1) Department of Molecular Medicine; National Public Health Institute, Helsinki, Finland; 2) Mental Health and Alcohol Research; National Public Health Institute, Helsinki, Finland; 3) Department of Human Genetics; University of California Los Angeles, Los Angeles, California, USA.

We have carried out a genome wide scan in a Finnish bipolar disorder family set. A total of 41 families were screened with 384 microsatellite markers covering the genome with a 9.3cM resolution. All the families contained at least two siblings with bipolar disorder type I or schizoaffective disorder, manic type defined by DSM-IV. We identified one distinct locus on 4q28.3 providing significant evidence of linkage in two-point analysis. Furthermore, a locus on 12q23.2 gave a two-point LOD score  $> 3.0$  and three loci with a LOD score  $> 2.0$  were observed with markers on 1q31.1, 16p11.1 and on Xq25, a locus already earlier identified in one extended Finnish pedigree (Pekkarinen et al. 1995). For a total of thirteen loci; 1q43, 2pq, 3p12.2, 3q13.31, 3q26.31, 5p12, 7p14, 8p22, 8q24.3, 9p21, 11pq, 14q21 and 14pq the two-point LOD scores exceeded 1.0.

Interestingly, regions on 1q31 ( $Z_{\max} = 2.3$ ; D1S1660), 4q28.3 ( $Z_{\max} = 3.3$ ; D4S1629), 5p13.3 ( $Z_{\max} = 1.6$ ; D5S1470), 12q23.2 ( $Z_{\max} = 3.0$  PAH), 16p11.1 ( $Z_{\max} = 2.9$ ; D16S769) have provided evidence for linkage also in previous studies from other populations (Detera-Wadleigh et al. 1999, Adams et al. 1998, Alda et al. 1998, Morissette et al. 1999, Craddock et al. 1994, Ewald et al. 1998, Dawson et al. 1995, Barden et al. 1998). We are currently finemapping these regions to obtain conclusive evidence for their involvement in the genetic background of bipolar disorder in Finland.

**Mapping of Susceptibility Loci for Vitiligo to Chromosomes 1p and 7.** A. Alkhateeb<sup>1</sup>, G.L. Stetler<sup>1</sup>, W. Old<sup>1</sup>, J. Talbert<sup>1</sup>, C. Miller<sup>1</sup>, P. Pengelly<sup>2</sup>, D.C. Bennett<sup>2</sup>, R.A. Spritz<sup>1</sup>. 1) Univ Colorado Heath Sci Ctr, Denver, CO; 2) Dept Anatomy & Cell Biol, St George's Hosp Med Sch, London, UK.

Vitiligo is a common, acquired, non-contagious disorder characterized by progressive, patchy depigmentation of the skin, overlying hair, oral mucosa, and occasionally the eyes, due to non-inflammatory loss of pigment-forming melanocytes from the affected areas, most likely on an autoimmune basis. Vitiligo is the most common disorder of pigmentation, with a frequency of ~1-3% in different populations. Familial clustering of cases is frequent, in non-Mendelian patterns indicative of a complex trait; ~20% of probands have at least one affected first-degree relative. We have carried out a genome-wide screen for linkage in four large Caucasian multiplex vitiligo kindreds, comprised of up to 14 affected unilineal individuals, selected to provide the greatest chance of detecting linkage. Genome-wide parametric linkage analysis gave indications of linkage at several positions, principally on chromosomes 1p and 7, with apparent locus heterogeneity among families. Non-parametric linkage analysis showed strong evidence of linkage to loci on chromosomes 1p and 7 in a subset of these families. Support for chromosome 1p linkage came principally from one family (14 affected, NPL=24.2,  $P=0.001$ ) and for chromosome 7 linkage from another family (9 affected, NPL=14.1,  $P=0.0003$ ). We then carried out a second non-parametric linkage study limited to chromosome 1 in six medium-sized Caucasian multiplex vitiligo kindreds. This analysis confirmed linkage to the same region of chromosome 1p in some of these families (overall NPL 8.92,  $P=0.0005$ ; HLOD 3.54,  $a=0.36$ ). Our results thus provide strong support for a locus on chromosome 1p, and likely another on chromosome 7, that confers susceptibility to vitiligo in a subset of multiplex families.

**Linkage Disequilibrium Mapping via Coalescent Modelling.** *D.J. Balding*<sup>1,2</sup>, *J.C. Whittaker*<sup>1,2</sup>, *A.P. Morris*<sup>2,3</sup>. 1) Dept Epidemiology/Public Hlth, Imperial Col Sch Medicine, London, England; 2) Dept Applied Statistics, University of Reading, England; 3) Wellcome Trust Centre for Human Genetics, Oxford, England.

Fine-scale linkage disequilibrium (LD) mapping using high-density SNP maps is widely recognised as having the potential to play a major role in identifying genes involved in complex traits. However, methods which analyse markers one at a time are inefficient, and so there is currently considerable interest in developing multipoint methods to fully exploit the information in linked SNP haplotypes. The success of a multipoint method depends on its ability to incorporate the effects of the genealogical history of the case chromosomes in the vicinity of a disease-predisposing mutation, including the effects of recombination, marker mutation, and demography (such as population growth). Most existing multipoint methods assume a star-shaped genealogy, which is an implausible scenario for the genealogical history. This can result in misleading inferences about the location of the disease mutation.

We have developed a Bayesian, Markov chain Monte Carlo method which explicitly models the underlying genealogical tree in the vicinity of a disease locus. Advantageous features of our method include allowing for uncertainty about both the true genealogy and the ancestral SNP haplotypes, allowing for phenocopies and more than one founding disease mutation event (at the same locus), and allowing for population growth. Simulation results suggest a substantial improvement over alternative multipoint LD mapping methods. Output from the method includes the approximate distributions of the location of the disease locus, of the age of the mutation, of the founding SNP haplotype, and of the allocation of case chromosomes into those bearing the most common disease-predisposing mutation, those bearing other mutations at the same locus, and phenocopies.

**Factor analytic methods in QTL mapping.** *L.E. Bauman<sup>1</sup>, J.S. Sinsheimer<sup>1,2</sup>, L. Almasy<sup>3</sup>, J. Blangero<sup>3</sup>, K. Lange<sup>1,2</sup>.*

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Variance component models decompose trait variances and covariances within and between pedigree members according to their sources: polygenic background, QTLs, shared environment and random environment. One can model the covariance matrix of a multivariate trait as a linear combination of Kronecker product matrices. Each Kronecker product combines a constant matrix such as a kinship matrix with an unknown matrix of parameters capturing the relationships between the various traits. Maximum likelihood estimation of these parameter matrices must obey complicated nonlinear constraints imposed by positive definiteness. It is possible to pose EM algorithms that finesse these constraints, but they tend to converge slowly. Alternatively, one can reparameterize each covariance matrix by its Cholesky decomposition. This leads to faster optimization algorithms. The Cholesky decomposition perspective also suggests factor analytic decompositions for investigating pleiotropy. Classical factor analysis seeks to explain the covariation between several traits by approximating the trait vector by a linear transformation of a small number of uncorrelated factors. Factor analysis has the potential for data reduction and for uncovering the coordinated control of multiple traits in QTL mapping. In the case of a pleiotropic QTL one would predict that the number of factors would be less than the number of traits. Such insight is more important than the exact nature of the factors. We have adapted our variance component program, FISHER, to perform maximum likelihood estimation using Cholesky and factor analytic decompositions. FISHER has performed well on preliminary application to a variety of data sets, both real and simulated, containing small and large pedigrees, highly correlated traits and QTLs showing pleiotropy. Time permitting, one or more applications will be discussed.

**Variance components linkage analysis of plasma amyloid b protein in typical late-onset Alzheimer's disease (LOAD) pedigrees.** *N. Ertekin-Taner<sup>1,2</sup>, J. Ronald<sup>1</sup>, N. Graff-Radford<sup>3</sup>, L.H. Younkin<sup>1</sup>, J. Adamson<sup>1</sup>, E. Atkinson<sup>4</sup>, M. deAndrade<sup>4</sup>, M. Baker<sup>1</sup>, M. Hutton<sup>1</sup>, S.G. Younkin<sup>1</sup>.* 1) Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Neuroscience, Mayo Clinic, Rochester, MN; 3) Neurology, Mayo Clinic, Jacksonville, FL; 4) Biostatistics, Mayo Clinic, Rochester, MN.

We previously showed that plasma Ab40 and Ab42 are significantly elevated in members of LOAD families compared to controls ( $p < 0.05$ ). To determine the magnitude of the genetic component affecting plasma Ab levels, heritabilities of plasma Ab42 and Ab40 were estimated in 16 extended LOAD pedigrees using variance components method. Heritability estimates of 62% and 49% were found for plasma Ab42 and Ab40, respectively. Bivariate analysis of these two quantitative phenotypes revealed a substantial genetic correlation between plasma Ab42 and Ab40 levels. Inclusion of the ApoEε4 dosage as a covariate did not have a significant effect on the heritabilities of these traits. These results suggest that genetic factors other than ApoE account for a substantial percentage of the phenotypic variance in both plasma Ab42 and Ab40 levels. Kehoe et. al. identified 5 chromosomal loci with multipoint lod scores  $> 3.0$  in the first stage of a genome-wide scan for LOAD in affected sib-pairs. We genotyped these candidate loci, in our independent collection of extended LOAD pedigrees, and performed linkage analysis, using plasma Ab levels as the surrogate phenotype. Evidence for linkage was detected to a locus on chromosome 10 at approximately 80 cM. Remarkably, significant linkage to the same region was obtained independently in the second stage of the LOAD sib-pair genome-wide scan that originally provided the candidate regions for our study. These results provide strong evidence for the existence of a novel LOAD locus on chromosome 10 that increases the risk for LOAD by increasing Ab levels. Our study also demonstrates that using plasma Ab as a surrogate quantitative phenotype may be a powerful approach to discover genetic loci for LOAD. Several candidate genes that reside in the linkage region are being screened for polymorphisms. Studies are underway to test the effects of these polymorphisms on plasma Ab levels and association with LOAD.

**QTLs on chromosomes 11 and 13 contributing to HDL-C levels and its subfractions in extended FCHL pedigrees.** *F. Gagnon, G.P. Jarvik, A.G. Motulsky, S.S. Deeb, J.D. Brunzell, E.M. Wijsman.* University of Washington, Seattle, WA.

A genome screen for HDL-C levels is in progress in 4 large (N=258) familial combined hyperlipidemia (FCHL) families, ascertained through hypertriglyceridemic probands. HDL-C is correlated with the lipid levels that define FCHL. Using joint linkage and segregation analysis based on Bayesian Markov chain Monte Carlo methods, we estimate that 3-4 genes are involved. Our preliminary results, focussing on chromosomes (ch) for which linkage to FCHL or HDL-C has been reported, provide evidence for HDL-C linkage to ch 11q and 13q. Several candidate genes, including the ApoAI-CIII-AIV complex, are in the critical region on 11q. From a joint multipoint analysis with all markers on ch 11 and 13, the posterior:prior probability of linkage (in a 2 cM interval and with a Poisson prior of 4 for the number of quantitative trait loci (QTLs)) is ~5 and ~8 for ch 11 and 13, respectively. Use of APOCIII as a major-gene covariate reduced evidence for linkage to 11q, supporting its role as an HDL-C locus. There is evidence for linkage to 11q and 13q for the HDL-C subfraction HDL3, whereas no or little evidence for linkage is observed for square root HDL2. We are following up on additional signals. These results confirm a previous report of linkage of HDL-C to 13q. Previous reports of linkage of FCHL to this ch 11 region have been published by others but results remain controversial and inconclusive. Ascertainment of families among studies varies with respect to procedures that could affect the HDL-C distribution, and thus could explain the controversial results obtained for linkage of FCHL to the 11q23 region.

**Finer linkage mapping of two regions harbouring suggestive susceptibility loci for osteoarthritis.** *K. Chapman<sup>1</sup>, Z. Mustafa<sup>1</sup>, B. Dowling<sup>1</sup>, B. Sykes<sup>1</sup>, A. Carr<sup>2</sup>, J. Loughlin<sup>1</sup>.* 1) IMM, University of Oxford, Oxford, UK; 2) Nuffield Orthopaedic Centre, Oxford, UK.

Osteoarthritis (OA) is a common late onset disease characterised by a degeneration of the articular cartilage in synovial joints. We have previously reported linkage to a number of chromosomes, principally chromosomes 2q, 4q, 6q, 11q and 16; using an affected sib pair approach. These regions were identified using marker panels of relatively low densities. The resulting linkages were thus very broad and required a finer linkage mapping approach before embarking on linkage disequilibrium/association analysis. To further assist with the finer linkage mapping, we expanded our family collection to 576 families comprising 1572 affected individuals who have undergone hip and/or knee joint replacement surgery due to severe OA.

We here report the refined linkage analysis of two of our regions, those on chromosomes 6q and 11q. In our original analysis of 6q, a broad linkage region was observed with over 50 cM of the chromosome having an MLS of 2.0 or greater. We have now refined this linkage to an interval of 25 cM, centred on markers located approximately 80 cM from the 6p-telomere, with a LOD = 3.0. The finer mapping of chromosome 11q has resulted in us narrowing the linkage peak to an interval of 15cM, centred on markers located approximately 77 cM from the 11p-telomere. This region, with a LOD = 2.0, is located 13 cM proximal to the original, crude 11q linkage. All multipoint linkage analysis was conducted utilising ALLEGRO v1.1. Both linkages are more pronounced in a subset of 150 families in which all the affected individuals are females with hip OA. Such gender and site-specific differences in the degree of OA susceptibility have been reported in epidemiological studies, and may assist in the choosing of appropriate, stratified cohorts for linkage disequilibrium/association analysis. (Supported by The Wellcome Trust and the Arthritis Research Campaign).



**A Locus for Elevated ApoB Levels in Familial Combined Hyperlipidemia.** *H. Allayee<sup>1, 2</sup>, K.L. Krass<sup>1, 2</sup>, P. Pajukanta<sup>1</sup>, R.M. Cantor-Chiu<sup>1</sup>, L. Peltonen<sup>1</sup>, J.I. Rotter<sup>1, 2</sup>, T.W.A. de Bruin<sup>3</sup>, A.J. Lysis<sup>1, 2</sup>.* 1) Dept. of Human Genetics, UCLA, Los Angeles, CA; 2) Dept. of Medicine, UCLA, Los Angeles, CA; 3) Dept. of Medicine, Maastricht University, Maastricht, the Netherlands.

Familial combined hyperlipidemia (FCHL) is a common genetic lipid disorder with a marked risk for premature coronary artery disease. Even though considerable effort has been put towards identifying FCHL susceptibility genes, the disease remains relatively uncharacterized at the genetic level. Various candidate gene studies in several populations and two genome scans in Dutch and Finnish families have been reported for the FCHL phenotype. While the latter studies each identified several loci, there were no obvious regions of overlap in the two genome scans. We speculated that this might be due to phenotypes not defined consistently across studies and to the application of different analytic methods. In order to permit an effective comparison between the two scans, we re-analyzed the genotype data in the Dutch FCHL families by employing the same methods that were used in the parametric linkage analyses of the Finnish families. In a parametric two-point genome scan of the Dutch families, significant evidence for linkage (Max LOD = 3.8 at  $\theta = 0$ ) of elevated apoB levels was observed at a locus on chromosome 1p (peak marker D1S1665). Additional markers in the region were genotyped and the most significant linkage result was obtained with a multipoint analysis of D1S1665 and D1S481 (Max LOD = 4.7). This locus is distinct from the previous FCHL locus identified on chromosome 1q in the Finnish families, although the 1q locus also yields some evidence for linkage in this Dutch sample (LOD = 1.8). Statistically significant evidence for linkage of elevated apoB levels to this new locus on 1p was not observed in the Finnish families, suggesting that FCHL in these two populations is a complex disorder that may be complicated by underlying genetic heterogeneity. The identification of this novel locus influencing apoB levels in this Dutch population may help to unravel the genetic complexity of FCHL.

**Genome scan for celiac disease in a Finnish founder family.** *N.J. Ali-Varpula<sup>1</sup>, P.M. Holopainen<sup>1</sup>, V. Ollikainen<sup>2</sup>, K. Mustalahti<sup>3</sup>, M. Maki<sup>3</sup>, J.A. Partanen<sup>1</sup>.* 1) Department of Tissue Typing, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland; 2) University of Helsinki, Finnish Genome Center, Helsinki, Finland; 3) Tampere University Hospital and Medical School, University of Tampere, Tampere, Finland.

Celiac disease (CD) is a multifactorial autoimmune-like disorder of the small intestine triggered by dietary gluten. The only established genetic risk factors are certain HLA DQ alleles on 6p21.3. Previous genome wide screenings for non-HLA susceptibility genes have suggested several loci with somewhat controversial results, pointing to linkage heterogeneity between populations. Genetic heterogeneity within the study sample can be diminished by concentrating on isolated founder populations. The population of Finland is known to feature strong founder effect. We performed a genome scan in an isolated Finnish subpopulation from the Northern part of Finland: 'Koilliskaira' founder families affected with CD. The families were genealogically confirmed to share an ancestor in 16th century. Genetic linkage between nine families with 23 CD patients and 399 markers was studied, using both multipoint parametric and non-parametric linkage analyses (NPL). A novel susceptibility locus with a significant maximum LOD score of 3.7 was found on chromosome 15q using a highly penetrant dominant model of inheritance. LOD score >2 was found on chromosome 8p, and LOD scores >1 on 2p, 4p, 5q, 12q, 15q and Xq. NPL scores >2 (nominal  $p < 0.01$ ) were obtained for 4p, 8p and 15q. By simulation approach, genome-wise significance for the NPL scores was not, however, reached in this small set of families. Surprisingly, no linkage with the known CD risk locus on chromosome 6p was found, although all patients carried the high risk allele HLA DQ2. The presence of genuine susceptibility factors on chromosomes 4p and 15q is further supported by our evidence from genome scan with an independent set of 60 unrelated Finnish CD families, in which linkage to these non-HLA regions was found. This points to relative homogeneity within this founder family, and its usefulness in genetic studies despite of small number of nuclear families.

**Chromosome 2q Linkage in Idiopathic Talipes Equinovarus (Clubfoot).** *S.H. Blanton<sup>1</sup>, S. Broussard<sup>4</sup>, C.A. Wise<sup>3</sup>, A. Scott<sup>2</sup>, J.T. Hecht<sup>4</sup>.* 1) Univ Virginia Health Sys, Charlottesville, VA; 2) Shriner's Hospital for Children, Houston, TX; 3) Scottish Rite Hospital, Dallas TX; 4) University of TX HSC, Houston.

Idiopathic talipes equinovarus (ITEV) is a common birth defect occurring in 1/1000 livebirths. Males are affected twice as frequently as females and either one or both feet can be involved. Treatment consists of serial casting and surgical correction and the final result is often sub-optimal. While the natural history of ITEV has been well characterized, little is known about the causal factors contributing to this common birth defect. Numerous studies have suggested that genetic factors contribute to the etiology. We have assembled a collection of Hispanic and non-Hispanic White probands in 275 parent-child trios (203 sporadic, 72 with a family history) and 48 extended multiplex families with ITEV. The region 2q31-q33 has been involved in a translocation in an ITEV patient and contains a number of candidate genes, including COL3A1, COL5A1, and ADAM23. DNA markers spanning this region were typed and the results analyzed using the TDT function of GENEHUNTER. For two of the markers, GATA149B10 alone (data shown below) and as a haplotype with D2S2979, a significant result was obtained in the sporadics and is strongest in the White non-hispanic sporadic trios.

Marker	Population	p-value	Permutation Test#
GATA149B10	All Sporadics	0.019	65
	White Sporadics	0.005	19

#indicates how many times out of 1000 would get the same p-value or better by chance.

These results clearly suggest that a gene in this region may play a role in the development of ITEV among sporadic non-Hispanic Whites. There are a number of candidate genes in this region in addition to the three mentioned above and we are evaluating each of them.

**Context-dependent linkage analysis of plasma cholesterol reveals its complex multifactorial etiology.** *K.L.E. Klos<sup>1</sup>, S.L.R. Kardia<sup>1</sup>, J.E. Hixson<sup>2</sup>, S.T. Turner<sup>3</sup>, E. Boerwinkle<sup>2</sup>, C.F. Sing<sup>1</sup>.* 1) Univ Michigan, Ann Arbor, MI; 2) Univ Texas Health Science Center, Houston, TX; 3) Mayo Clinic, Rochester, MN.

Interindividual variation in a quantitative trait that influences the risk of developing a common disease, such as coronary artery disease (CAD), is expected to be influenced by gene-gene and gene-environment interactions. Plasma total cholesterol (TC) is known to be correlated with CAD risk factors that are influenced by genetic and environmental variations. We carried out variance components-based linkage analysis to identify regions of the genome influencing variation in TC within subsets of pedigrees which differed in genetic and environmental context as indexed by BMI, triglycerides (TG) and HDL-cholesterol (HDL-C). Each trait was used to subdivide pedigrees into high and low subsets. Within these subsets a second trait was used to further subdivide pedigrees. Thirty-eight markers were selected approximately every 100cM to represent variation in the genetic background among subsets of pedigrees. For all methods of subdivision, 9.2% of background markers differed significantly ( $p < 0.01$ ) in % heterozygous individuals among subsets. Because subsets may differ in the information content of markers at some genomic locations, we examined specific markers near LOD peaks  $> 2.00$  for % heterozygous individuals among subsets, eliminating one peak on chromosome 20 from further consideration. Within particular subsets evidence remained of linkage with genes influencing TC in 6 regions involving chromosomes 3, 4, 11, 13 and 17. For example, a LOD score of 2.77 in pedigrees with high TG suggests the presence of a gene(s) influencing TC on chromosome 11. A LOD score of 3.21 was observed on chromosome 13 in the subset of pedigrees defined by low BMI and high HDL-C. Two regions of chromosome 17 may contain genes which influence TC under different contexts. We obtained LOD scores of 2.45 on the long arm in pedigrees with high HDL-C, and of 2.26 on the short arm in pedigrees with both high HDL-C and high TG. Our findings further document the importance of context in modeling the relationship between genetic and quantitative phenotypic variation. Supported by NHLBI grant HL39107.

**Linkage between cholesterol 7  $\alpha$ -hydroxylase (CYP7) gene and low-density lipoprotein cholesterol (LDL-C) in increased CHD risk Caucasian families - the NHLBI Family Heart Study.** *J.P. Lin<sup>1</sup>, P. Sholinsky<sup>1</sup>, H.H. Coon<sup>2</sup>, S.C. Hunt<sup>3</sup>, R.H. Myers<sup>4</sup>, D.K. Arnett<sup>5</sup>, Y. Hong<sup>6</sup>.* 1) Division of Epidemiology and Clinical Applications, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD; 2) Department of Psychiatry, University of Utah, Salt Lake City, UT; 3) Department of Internal Medicine, University of Utah, Salt Lake City, UT; 4) Section of Preventive Medicine, Boston University School of Medicine, Boston, MA; 5) Division of Epidemiology, University of Minnesota, Minneapolis, MN; 6) Division of Biostatistics, Washington University School of Medicine, St. Louis, MO.

Genetic factors account for approximately 50% of the individual variation in plasma LDL-C concentrations in the general population. Several candidate genes have been proposed but their relative contributions to the variance in LDL-C are not clear. In a recent study of a randomly sampled population, the allelic variation in the CYP7 gene explained 15% of the phenotypical variation in plasma LDL-C concentrations. In this report we present linkage results between LDL-C and CYP7, as well as apolipoprotein E (APOE) and low-density lipoprotein receptor (LDLR), three pivotal genes in LDL metabolism. Our study population included more than 200 Caucasian nuclear families with increased coronary heart disease (CHD) risk from the NHLBI Family Heart Study. Individuals on medication for high cholesterol, or with Familial Combined Hyperlipidemia, or triglycerides > 300mg/dl were excluded. Haseman-Elston sibling pair (S.A.G.E. 4.0-7) and variance-component (SOLAR) linkage methods were used. The results showed significant linkage between plasma LDL-C concentrations and CYP7 ( $p=0.007$  for Haseman-Elston;  $p=0.017$  for variance-component). The allelic variation in CYP7 accounts for approximately 30% of the phenotypical variation in plasma LDL-C concentrations. When the analyses were repeated on the APOE and LDLR loci, no evidence of linkage was detected. These findings suggest that polymorphism in CYP7 contributes importantly to the variation in plasma LDL-C concentrations in a population at increased risk of CHD.

**A permutation procedure for the haplotype method for identification of disease-predisposing variants.** *H. Li.*  
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Once a genetic region involved in a complex disease has been localized through linkage or association studies, we need methods to help us identify the actual disease predisposing genetic variant(s) in the region. A large number of singlenucleotide polymorphic (SNP) sites may exist in this region. It is important to identify genetic variants directly involved in disease from those in linkage disequilibrium, and thus associated with, the disease predisposing variant(s). A question of great interest is to test whether a SNP or a combination of SNPs that influence the trait under investigation has been identified. For many complex HLA-associated diseases, patterns of amino acid site variability raise the possibility that HLA-variation association with a disease may not be due to a given allele but rather one or more variable amino acid sites occurring on several alleles. Here the question is whether an amino acid variant or a combination of amino acid variants involved in disease is identified. To address this question, this paper proposes a permutation procedure for the haplotype method to test whether all the sites involved in the disease have been identified using the haplotypic data of patients and controls. The method is based on the theoretical result of Valdes and Thomson, that, for each haplotype combination containing all the amino acid sites involved in the disease process, the relative frequencies of amino acids variants at sites not involved in disease, but in linkage disequilibrium with the disease-predisposing sites, are expected to be the same in patients and controls. This procedure takes into account the non-independence of the sites sampled and is robust to mode of inheritance and penetrance of the disease, and can definitely specify when all the disease predisposing sites have not been identified. Application to both simulated data and real data sets on type 1 diabetes and alcoholism indicates that the proposed procedure works well in testing the important null hypothesis of whether all the predisposing sites are identified.

**Genome-wide scan for type 2 diabetes mellitus in Japanese.** *N. Iwasaki<sup>1</sup>, X. Wang<sup>2</sup>, P. Schwalz<sup>2</sup>, M. Honda<sup>3</sup>, M. Saito<sup>4</sup>, N. Kamatani<sup>4</sup>, N.J. Cox<sup>2</sup>, G.I. Bell<sup>2</sup>, Y. Iwamoto<sup>1</sup>.* 1) Diabetes Center, Tokyo Women's Medical Univ., Shinjuku-ku, Tokyo, Japan; 2) Howard Hughes Medical Institute, The University of Chicago, Chicago, IL; 3) Shiseikai Daini Hospital, Tokyo, Japan; 4) Institute of Rheumatology, Tokyo Women's Medical Univ., Shinjuku-ku, Tokyo, Japan.

**Background and Aims:** Genetic factors contribute to the development of type 2 diabetes mellitus. We have carried out a genome-wide screen for type 2 diabetes genes in the Japanese population, as a first step in the identification of diabetes-susceptibility genes in this population. **Materials and Methods:** The study population consisted of 256 affected sib pairs (379 diabetic subjects from 164 families) recruited from among the patients attending the Diabetes Clinic of Tokyo Women's Medical University and associated hospitals. The clinical features of the study population are: age-at-diagnosis, 45.3±10.9 years (mean±SD); HbA1c, 7.7±1.74 % and BMI, 23.0±3.0 kg/m<sup>2</sup>. DNA was prepared from blood and genotyped using a panel of 414 markers from chromosomes 1-22. We tested each marker for linkage with type 2 diabetes using both two-point (programs SIBPAIR and SPLINK) and multipoint (GENEHUNTER-PLUS) methods of analysis. **Results:** We found 9 regions that showed nominal multipoint evidence of linkage with type 2 diabetes (i.e. MLS > 0.59, P<0.05). These regions were on chromosomes 1p(25cM), 2q (152-167 cM), 4q (92 cM), 5q (114cM), 6p (33 cM), 8p (4cM), 9p (0-28 cM), 17p (34cM) and 21 (39-52 cM). One region of chromosome 9 (3-21 cM) showed suggestive evidence for linkage with MLS = 3.5 when families are weighted according to the number of (pairwise) independent affected sib pairs. **Conclusions:** Our genome-wide screen has revealed possible locations of genes that may contribute to the development of type 2 diabetes in the Japanese population. These results provide a focus for further genetic studies of type 2 diabetes in Japanese and the identification of genes that increase the risk of type 2 diabetes in this population.

**Detection of genetic heterogeneity among families with multiple affected relatives.** *L. Mirea, L. Briollais, S.B. Bull.* Public Health Sciences, University of Toronto and SLRI, Mount Sinai Hospital, Toronto, ON.

Linkage analyses based on allele-sharing in sets of affected relatives are less powerful when heterogeneity exists among the allele-sharing distributions of sampled families. The presence of genetic heterogeneity may be indicated by variability in symptoms, prevalence, and/or age of onset for the disease. To test for heterogeneity, we propose using family-level binary covariates and family-specific non-parametric linkage scores (NPL). In one approach we extend the linkage likelihood of Kong and Cox (1997, *Am J Hum Genet* 61:1179-1188) to include a family indicator covariate and formulate a likelihood ratio test (LRT) and efficient score test (ESS). Alternatively, asymptotic (T) and permutation methods (Tperm) are applied to assess differences in mean NPL scores between two covariate-defined family subgroups. We investigated these approaches using simulated marker genotypes (10 equally frequent alleles) for samples of 150 families with affected sibling or cousin pairs generated under dominant and recessive inheritance models. Family subgroups were defined by a binary covariate at levels 50%, 60% and 80%. Homogeneous samples included only linked families ( $q=0$ ), whereas covariate values precisely identified linked ( $q=0$ ) and unlinked ( $q=0.5$ ) families within heterogeneous samples. The type I error rate of ESS was grossly inflated while those of LRT and T were for the most part conservative. Additional simulations showed that in the absence of heterogeneity when linkage was either absent or very strong, the empirical distributions of LRT, ESS and T did not follow that specified by standard asymptotic theory. In all scenarios examined, Tperm was found to have a nominal 5% type I error and power in excess of 90% which was comparable to that calculated for LRT, ESS and T using adjusted critical values. The power of all methods decreased more rapidly for the recessive model as the difference between subgroup sizes increased. We recommend the use of Tperm but caution that power will decrease when the covariate selected to stratify families is subject to misclassification.



**Characterisation of a Major Locus for Developmental Dyslexia on 18p11.2.** *I.L. MacPhie<sup>1</sup>, S.E. Fisher<sup>1</sup>, C. Francks<sup>1</sup>, A.J. Marlow<sup>1</sup>, L.R. Cardon<sup>1</sup>, J.B. Talcott<sup>2</sup>, A.J. Richardson<sup>2</sup>, J.F. Stein<sup>2</sup>, A.P. Monaco<sup>1</sup>.* 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 2) Department of Physiology, University of Oxford, UK.

Developmental dyslexia is a difficulty in learning to read that can not be explained by deficits in intelligence, motivation, educational opportunity, or sensory acuity. It is one of the most frequently diagnosed learning disabilities, estimated to affect between 5 and 10% of school-aged children, and represents a major educational, social and mental health problem. Previous work has clearly shown dyslexia to be a significantly heritable trait, but the genetic etiology remains largely unknown. We recently reported the first complete QTL-based genome-wide scans for dyslexia in two large, independent samples from the UK and the US (Fisher et al, submitted). A highly significant novel QTL influencing multiple reading-related processes was detected yielding top multipoint p values < 0.0003 in both samples. Moreover, this linkage was replicated in a third sample of families from the UK. Using the public sequence databases we have constructed a transcript map of the region between D18S452 and D18S478. The relative scarcity of genes around 18p11 allowed us to identify a small number of candidates between these markers for further analysis. Probands were screened for gene-specific polymorphisms using D-HPLC and the observed variants were then followed up by direct sequencing and tested for association using QTDT. Refinement of the 18p11.2 QTL is an important step towards identifying susceptibility genes for this complex trait and elucidating the molecular mechanisms behind reading disability.

**Liability loci for multifactorial cleft lip and palate map to the same chromosomal locations as liability loci for multifactorial neural tube closure defects in mice.** *D.M. Juriloff, M.J. Harris, T.M. Gunn, M.K. Wu, D.G. Mah, S.L. Dewell.* Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada.

The A/WySnJ mouse strain has about 20% cleft lip with or without cleft palate, CL(P), of genetically multifactorial cause. After a cross to C57BL/6J, we used CL(P) embryos from 1,485 BC1 segregants in a genome screen to map the major liability loci, *clf1* and *Clf2*, to Chr 11 near *D11Mit10* and to Chr 13 near *D13Mit13*. Another strain, SELH/Bc, has 10-30% exencephaly, a cranial neural tube closure defect (NTD), of genetically multifactorial cause. After a cross to the normal LM/Bc strain, we used the extremes of 102 testcrossed F2 males in a genome screen to map the major liability loci. The chromosomal regions identified were confirmed in 31 exencephalic F2 segregants and were further confirmed in 24 exencephalic embryos from 758 F2 segregants from a cross of SELH/Bc with the JAX "curly tail" strain. The confirmed exencephaly liability loci mapped to Chr 13 near *D13Mit13* and to Chr 11 near *D11Mit10*, the same regions as the previously mapped CL(P) liability loci. The homologous human linkage groups are located on 17q21 and 5q or 9q. For CL(P), *clf1* is recessive, *Clf2* is semidominant, and the loci interact epistatically. For exencephaly, the risk alleles are semidominant and additive across loci. Each defect is strain specific.

The unexpected convergence of the map locations of the major liability loci for CL(P) and exencephaly from two mouse strains suggests that different alleles at the same loci may be involved in both traits. Our data therefore suggest that human multifactorial NTD and multifactorial CL(P) may share genetic factors in their etiologies.

**TCRG - a positional and functional candidate for asthma: linkage and association analysis among the Finns.** *T. Laitinen*<sup>1</sup>, *A. Polvi*<sup>1</sup>, *P. Sevon*<sup>2</sup>, *J. Vendelin*<sup>1</sup>, *P. Kauppi*<sup>3</sup>, *T. Petäys*<sup>4,5</sup>, *T. Haahtela*<sup>4,5</sup>, *L.A. Laitinen*<sup>3</sup>, *J. Kere*<sup>2</sup>. 1) Dept Medical Genetics; 2) Finnish Genome Center; 3) Dept Pulmonary Medicine; 4) Dept Allergological Diseases, Univ Helsinki; 5) Dept Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland.

Recent genome-wide scans have suggested several regions potentially linked to asthma and atopy related traits. One of the loci of interest is located on 7p15-p14. Among Finnish asthma families, this locus was the only locus that exceeded the threshold for genome-wide significance. The finding was further confirmed in two independent data sets, French-Canadians and North-Karelian Finns. As a potentially interesting candidate, the T cell receptor gamma genes (TCRG) has been mapped into this region. TCRG spans ~100kb containing several genes known to be highly polymorphic and rearrange during gamma/delta T cell maturation. Gamma/delta T cells are believed to form the first line of defense on epithelial surfaces and be involved in the development of immune disorders. We screened altogether 11 dinucleotide repeats to find new informative and stable markers in the TCRG locus, and three showed to be polymorphic. Linkage analysis of the TCR markers and one flanking marker (D7S2497) was performed among the Finnish genome scan pedigrees (N=86). The result showed that TCRG is located within the linkage region, but the observed linkage (NPL 3.11, P=0.001) did not exceed the previously found linkages (NPL 3.9, P=0.0001) for the markers upstream to TCRG. Haplotype analysis was done using all the chromosomes retrieved from multiplex and a new set of nuclear families (N=83, a total of 468 chromosomes). Haplotype associations were detected, but they all remain weak (chi-square<6.9). To study if the overall haplotype distribution in TCRG differs from expected among the disease associated compared to control chromosomes, we used Haplotype Pattern Mining. Again, no statistically significant bias in haplotype distribution was found. Our initial results on the TCRG locus suggest that even though TCRG is located in the linkage region, it may not be the genetic regulator of asthma related traits among the Finnish patients.

**Modulation of severity in multiple sclerosis by polymorphisms in and around APOE.** *J.L. Haines<sup>1</sup>, S. Schmidt<sup>2</sup>, L.F. Barcellos<sup>3</sup>, K. deSombre<sup>2</sup>, R.R. Lincoln<sup>3</sup>, P. Bucher<sup>3</sup>, E.R. Martin<sup>2</sup>, E. Lai<sup>4</sup>, A.M. Saunders<sup>2</sup>, J.M. Vance<sup>2</sup>, J.R. Oksenberg<sup>3</sup>, S.L. Hauser<sup>3</sup>, M.A. Pericak-Vance<sup>2</sup> and Multiple Sclerosis Genetics Group.* 1) Program in Human Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Center for Human Genetics, Duke Univ Med Ctr, Durham, NC; 3) Dep't Neurology, Univ Cal San Francisco, San Francisco, CA; 4) Glaxo/SmithKline, Resarch Triangle Park, NC.

Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system with a complex etiology. While the contribution of the Major Histocompatibility Complex (MHC) to genetic susceptibility for MS has been established, identifying other risk genes has been problematic. Numerous studies have pointed toward the region on chromosome 19q13 surrounding the APOE gene as likely to harbor an MS susceptibility gene. In addition to gene discovery for MS risk genes in this region, several clinical reports have suggested that the APOE-4 allele may be associated with more severe disease and significantly faster progression of disability in MS. To examine the role of APOE, we genotyped APOE and seven other single-nucleotide polymorphisms (SNPs) located in a surrounding 920 kb region in a data set of 398 families, of which 148 are multiplex (2+ affected family members) and 250 are simplex. Most of these SNPs are within 16 kb of APOE and spaced 2-5 kb apart. Using family-based association analysis, we found statistically significant evidence that APOE itself or a gene in strong linkage disequilibrium with APOE increases susceptibility to MS in the data set of simplex families ( $p=0.002$  for a three-marker haplotype including the APOE-4 allele) but not multiplex families. Analysis of clinical variables indicated that APOE-4 carriers are more likely to have severe disease ( $p=0.03$ ), whereas APOE-2 carriers are more likely to exhibit mild disease ( $p=0.02$ ). This was true in both the multiplex and simplex families. These results suggest that APOE and/or nearby genes may modulate both risk and severity of MS.

**Total genome-scan and dense mapping in search for susceptibility genes in the type I allergy phenotypes: Atopic dermatitis, allergic rhinitis and allergic asthma.** A. Haagerup<sup>1</sup>, A.D. Borglum<sup>1</sup>, H.G. Binderup<sup>1</sup>, T.A. Kruse<sup>2</sup>. 1) Institute of Human Genetics, Aarhus University, Aarhus, Aarhus C, Denmark; 2) Clinical Institute, University of Odense, Odense, Denmark.

Through the ITA-project (Inheritance of Type I Allergy) we sampled 100 nuclear families (N=424) selected by affected sibpairs. Half the families (panel A) qualified for a comprehensive total genome-scan using a set of 446 highly informative microsatellite markers by holding sibpairs with both clinical disease and documented atopy, i.e. RAST > 1+. The other half of the families (panel B) contained clinical affected sibpairs with only one or no RAST positive sibling. The data from the genome-scan was analysed by non-parametric multipoint linkage analysis using MAPMAKER/SIBS and revealed evidence of both major and minor loci for most phenotypes. Best LOD scores were 2.79, 3.26, 2.66, 2.13, and 2.61 for atopic dermatitis (AD), allergic rhinitis (AR), allergic asthma (AA), atopy (RAST > 1+), and elevated total IgE (s-IgE > 100 kU/i), respectively. For a second scan candidate regions on chromosome 3, 4, 5, 6, 9, 12, 18, and X were selected for dense mapping. We used 97 microsatellite markers and analysed all 100 families. LOD scores were computed in both panel A and in the total sample (panel A+B) for the five phenotypes. Evidence for linkage in several candidate regions rose whereas other regions could be discharged. Now candidate genes in the appointed regions are being further investigated.

**Large-scale candidate gene screening and association analysis in schizophrenia.** *N. Norton<sup>1</sup>, N.M. Williams<sup>1</sup>, H.J. Williams<sup>1</sup>, G. Spurlock<sup>1</sup>, N.J. Bray<sup>1</sup>, S. Jones<sup>1</sup>, G.S. McCarthy<sup>1</sup>, G. Jones<sup>1</sup>, S. Zammit<sup>1</sup>, A. Cardno<sup>1</sup>, R. Owen<sup>1</sup>, K.L. Davis<sup>1</sup>, J.D. Buxbaum<sup>2</sup>, V. Haroutunian<sup>2</sup>, M.J. Owen<sup>1</sup>, M.C. O'Donovan<sup>1</sup>.* 1) Neuropsychiatric Genetics, UWCM, Cardiff, S. Glamorgan, UK; 2) Department of Psychiatry, Mount Sinai School of Medicine, New York.

We have screened over 50 candidate genes for schizophrenia for sequence variation using DHPLC. To date, we have identified more than 100 SNPs. In contrast to most sequence variants available in public databases, we have confirmed every reported SNP by a secondary assay, and estimated allele frequencies in DNA pools by either DHPLC analysis or SNaPshot. DNA pooling with SNaPshot allowed allele frequencies to be estimated in up to seven SNPs in multiplex. When compared with individual genotyping, the mean error in estimating the absolute allele frequencies is 0.035 (SD=0.013, maximum = 0.037). More importantly, the mean error for estimating differences between cases and controls (assuming 100% accuracy for individual genotyping) was 0.006 (SD=0.008, maximum = 0.023). Our approach to candidate gene analysis is now to: 1) estimate allele frequencies in case-control pools of schizophrenic and control subjects using SNaPshot; 2) undertake individual genotyping where estimated p value is <0.1; 3) extend analysis to family based association samples. In this presentation, we summarize the results of such analyses of 57 candidates for schizophrenia.<

**Chromosome 19 and atopic asthma in Italian families.** *G. Malerba<sup>1</sup>, M.C. Lauciello<sup>1</sup>, R. Galavotti<sup>1</sup>, E. Trabetti<sup>1</sup>, A.L. Boner<sup>2</sup>, P.F. Pignatti<sup>1</sup>.* 1) Mother-Child & Biol, Genetics, Univ Verona, Verona, Italy; 2) Mother-Child & Biol, Paediatrics, Univ Verona, Verona, Italy.

DNA markers on chromosome 19 have been described to be linked to atopic asthma or related phenotypes in several populations. We have recently reported suggestive linkage of 19q31 DNA markers to atopy or skin prick test positivity (SPT) in a sample of Italian families and we suggested a potential linkage to chromosome 19p with atopy, SPT or total serum elevated IgE (IgE)(Venanzi et al., Clin Exp Allergy 2001 in press). Candidate gene analysis in the 19q region provided no evidence for linkage or transmission disequilibrium for marker IL11. The families have been genotyped with 9 additional DNA microsatellite markers spanning the 19p chromosomal region from D19S591 to D19S884 markers (about 18 cM). Five binary traits were investigated: asthma, SPT, IgE, bronchial hyperresponsiveness to methacholine and atopy. Non parametric pairwise linkage analysis and transmission disequilibrium test (TDT) were performed. Linkage was observed with D19S536 (IgE:p=0.007, asthma:p=0.024), D19S216 (IgE:p=0.04), D19S567 (asthma:p=0.021) and D19S884 (IgE:p=0.037). A significant TDT was observed for D19S120 (IgE:p=0.018), D19S567 (atopy:p=0.037, SPT:p=0.002), FcepsilonR2 (atopy:p=0.037) and D19S534 (IgE:p= 0.004) markers. These results suggest that a gene related to atopic phenotypes maps on chromosome 19p.

**Association of the DBH TaqI A polymorphism with Attention Deficit Hyperactivity Disorder in the Milwaukee Longitudinal Study of ADHD.** *B.A. Navia<sup>1,2</sup>, K.M. Smith<sup>2</sup>, L. Bauer<sup>3</sup>, M. Fischer<sup>3</sup>, M.J. Daly<sup>4</sup>, E. Lander<sup>4</sup>, R. Barkley<sup>5</sup>.* 1) Neurology, NEMC/Tufts Univ. Sch. of Medicine, Boston, MA; 2) Genetics Prg., Sackler School, Tufts Univ., Boston, MA; 3) Neuropsych. Sect., Med. College of Wisconsin, Milwaukee, WI; 4) Whitehead Institute, MIT, Cambridge, MA; 5) Dept. of Psychiatry, UMass Medical Center, Worcester, MA.

Attention Deficit Hyperactivity Disorder (ADHD) is a highly heritable and common disorder that partly reflects alterations in dopamine function. Genetic studies have shown that genes involved in dopamine signaling and metabolism, the 7 repeat allele of the D4 dopamine receptor (DRD4) exon 3 VNTR, the 480 bp allele of the Dopamine Transporter (DAT1) VNTR, and the Dopamine b Hydroxylase (DBH) TaqI A2 allele polymorphism contribute to ADHD susceptibility. We have initiated genetic studies of candidate genes for ADHD in a unique cohort of 158 ADHD and 81 control participants from the Milwaukee Longitudinal Study (MLS) of ADHD. Participants were diagnosed during early childhood, and have been evaluated every 5 years for the past 20 years. Evaluations have included neuropsychological, psychiatric, educational, and social functioning assessments. Eighty-eight Caucasian subjects and 40 age and ethnicity-matched controls from the MLS were genotyped for the DRD4 exon 3 VNTR, DAT1 3' UTR VNTR, DBH TaqI A polymorphism, and the DBH GT repeat polymorphism which has been previously associated with serum DBH activity levels. Results indicate a significant association between the DBH TaqI A1 allele and ADHD ( $p < .01$ ) with a greater number of individuals with the A1, A1 genotype in the ADHD group ( $p < .005$ ). Also, a trend towards an increased frequency of the DBH GT repeat A4 allele in the ADHD group ( $p < .25$ ) was observed. These data support the hypothesis that particular alleles of the DBH gene may confer susceptibility to ADHD. Further studies will aim to genotype other candidate alleles in these individuals and in the full MLS cohort. These data will allow the potential to study the functional phenotypic consequences of candidate alleles on different developmental outcomes of ADHD.



**Chromosome 12 and Late-Onset Alzheimer Disease (AD) is Modified by Apolipoprotein E (APOE) Genotype.**

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There are several reports of association or linkage between chromosome (chr) 12 markers and AD but there is no consensus for any specific candidate gene. Since the APOE-4 allele is a strong risk factor for AD, we examined whether association between markers on chr 12 and AD is modulated by APOE genotype. We assessed both linkage and association in 251 Caucasian families with discordant sibships and mean age of onset of AD per family > 60 years. The markers spanned a region from 26 to 80 cM. Data were analyzed using two family-based tests of linkage disequilibrium: the pedigree disequilibrium test using (PDT), and TRANSMIT (TMT). We report results of 2-point single-allele tests with 1 df. In the overall dataset, results were significant for the 234-bp allele of d12s1090 (56 cM; p=0.002 TMT; p=0.01 PDT), and for the 175-bp allele of d12s1713 (61 cM; TMT p=0.001; PDT ns). The 234 bp allele at D12S1090 was also associated with AD (p=0.003 TMT; 0.01 PDT) in families where all affected individuals had at least one APOE-4 allele (n=94). However, several novel associations were detected in families where at least one affected individual had an APOE 3/3 genotype (n=67): 218-bp allele of d12s1632 (71.6 cM; p=0.001TMT; p=0.03 PDT), the 186-bp allele of d12s2200 (79.9 cM; p=0.02 TMT; PDT ns) and the 210-bp allele of d12s2200 (p=0.01 TMT; PDT ns). We further examined these APOE-4 independent results in families where all affected members carried the APOE 3/3 or 3/2 genotypes (n=37). Association was found with the 218-bp allele of d12s1632 (p=0.03 TMT; PDT ns); with the 202-bp allele of D12S2200 (p=0.003 TMT; p=0.03 PDT); and with the 175-bp allele of d12s1713 (p=0.0006 TMT; p=0.01 PDT). These data indicate that association between markers on chr 12 and AD is dependent on APOE genotype, and that more than one AD susceptibility gene may exist on chr 12.

**Juvenile myoclonic epilepsy in chromosome 6p11: Recombinations and haplotype sharing reduce the candidate region to 500 kb.** *R. Morita*<sup>1,2</sup>, *D.S. Bai*<sup>2</sup>, *M.E. Alonso*<sup>3</sup>, *T. Suzuki*<sup>1</sup>, *Y. Sugimoto*<sup>1</sup>, *K. Amano*<sup>1</sup>, *M.T. Medina*<sup>2,5</sup>, *J.N. Bailey*<sup>2,6</sup>, *A. Rasmussen*<sup>3</sup>, *J. Ramos-Peek*<sup>3</sup>, *S. Cordova*<sup>3</sup>, *F. Rubio-Donnadieu*<sup>3</sup>, *J. Inazawa*<sup>4</sup>, *A. Jara-Prada*<sup>3</sup>, *A. Ochoa*<sup>3</sup>, *K. Yamakawa*<sup>1</sup>, *A.V. Delgado-Escueta*<sup>2</sup>. 1) Lab Neurogen, Brain Sci Inst, Inst Physical & Chemical Res, Saitama, Japan; 2) Epilepsy Genetics/Genomics Lab, CEP, UCLA and VA GLAHS, West Los Angeles, CA; 3) National Institute of Neurology & Neurosurgery, Mexico City, Mexico; 4) Dept Mol Cytogenetics, Div of Genetics, Med Res Inst, Tokyo Med & Dental Univ, Tokyo, Japan; 5) Autonomous Univ, Tegucigalpa, Honduras; 6) Neuropsychiatric Inst, UCLA, Los Angeles, CA.

Juvenile myoclonic epilepsy, which accounts for 12-30 percent of all epilepsies, starts at 13-15 years of age. Grand mal seizures appear two years later. In 1988, we reported a major susceptibility loci for JME (EJM1) may lie outside the HLA area in chromosome 6p21.3. In 1995-1996, a large family from Belize provided independent proof for linkage to 6p12-11 about 25 cM centromeric to HLA. To reduce the size of the candidate region for EJM1, we constructed a PAC and BAC based physical map composed of 95 clones to order microsatellite markers accurately, and generated six new microsatellites from clone sequences in the EJM1 candidate region. We studied a second large family from Appalachian mountains (USA) that provided independent proof of linkage (LOD>3) to 6p12-11. Informative recombinations and haplotypes in these two large families reduced the size of EJM1 to 500kb flanked by 125gt1 and 408CA1. Linkage analyses, haplotypes and recombinations in 21 other medium-sized JME families (243 individuals; 77 affected) from Mexico and California provided supporting evidence for the EJM1 locus. Pooled LOD scores were significant, e.g., 4.73 for D6S1662 on two point linkage analyses. No evidence of linkage was observed for markers in HLA region. Haplotype analyses identified 14 families whose affected members shared a one megabase 6p11 region flanked by AFMA053WF1 and D6S1960 while recombinations identified the same 6p11 area where 125gt1, D6S427 and 408CA1 reside. Large scale sequencing of this 550kb area is in progress.

**Genetic mapping using haplotype and model-free linkage analysis supports previous evidence for a locus predisposing to severe bipolar disorder at 5q31-q33.** *K.S. Hong<sup>1,6</sup>, A. McInnes<sup>2</sup>, S. Service<sup>1</sup>, T. Song<sup>2</sup>, J. Lucas<sup>2</sup>, S. Silva<sup>3</sup>, E. Fournier<sup>3</sup>, P. León<sup>3</sup>, J. Molina<sup>4</sup>, V.I. Reus<sup>2</sup>, L. Sandkuijl<sup>5</sup>, N.B. Freimer<sup>1</sup>.* 1) Department of Neuropsychiatry, University of California Los Angeles, Los Angeles, CA; 2) Department of Psychiatry, University of California San Francisco, San Francisco, CA; 3) Cell and Molecular Biology Research Center and Escuela de Medicina, Universidad de Costa Rica, San Jose, Costa Rica; 4) Center for Regional Investigations in Mesoamerica, Antigua, Guatemala; 5) Department of Human Genetics, Leiden University, The Netherlands; 6) Department of Psychiatry, Sungkyunkwan University School of Medicine, Seoul, Korea.

In earlier work (Garner et al., 2001) we screened the genome of two separately ascertained but genetically connected Costa Rican pedigrees segregating for severe bipolar disorder (BPI). In that analysis, we had combined the two pedigrees as a single huge kindred and used recently developed algorithms permitting non-parametric linkage analysis of large, complex pedigrees with inbreeding loops (implemented in the package SimWalk2) and obtained strong evidence for linkage to BPI with five microsatellite markers in 5q31-33. In the present study, we genotyped 14 new microsatellite markers in this region on chromosome 5 and applied the same model-free linkage analysis. Significantly high degree of identity-by-descent allele sharing among affected relatives was observed in all the markers spanning approximately 15cM ( $p < 0.01$ ). We also constructed haplotypes with these markers for 74 individuals in the pedigree. Twelve of 19 BPI affected individuals in the larger branch of the pedigree shared a single haplotype over this region, with several more affected individuals sharing at least some parts of this haplotype. These results support the suggestion for a locus at 5q31-q33, that together with a previously reported locus at 18q22-q23, provides the major genetic risk for BPI in these pedigrees.

**First results of a genome-wide scan in Dutch Caucasian sibpairs with type 2 diabetes mellitus.** *J.H.O. van Tilburg<sup>1</sup>, E. Strengman<sup>1</sup>, P.L. Pearson<sup>1</sup>, L.A. Sandkuijl<sup>1</sup>, T.W. van Haefen<sup>2</sup>, C. Wijmenga<sup>1</sup>.* 1) Department of Medical Genetics, University Medical Center, Utrecht, the Netherlands; 2) Department of Internal Medicine, University Medical Center, Utrecht, the Netherlands.

Type 2 diabetes mellitus is increasingly common in adults in most populations in the western world. In the past two decades, twin and family studies have provided strong evidence for a genetic component in diabetes. It is now well established that diabetes mellitus is a multifactorial disease, in which many genes interact not only with each other but also with environmental factors. Factors such as age, physical activity, diet, and obesity play a major role in the disease aetiology.

We are currently performing a genome-wide scan in Dutch Caucasian sibpairs with type 2 diabetes mellitus (DM2), with the aim of identifying susceptibility loci in the Dutch population. Patients were recruited through the Diabetes Service in Breda, and diagnosed according to WHO criteria. So far, our study cohort consists of 586 probands, including 336 sibpairs with a mean sibship size of 3.1 .

We use a screening set of approximately 300 highly polymorphic markers, giving an average spacing of 15 cM. This set will provide a power of  $\pm 90\%$  to detect loci of even modest effect ( $l_s = 1.5$  to  $1.7$ ). The screening set is designed in such a way that multiplex PCR can be performed. The PCR products are pooled into different running sets, before electrophoresis on an ABI 3700, and are analysed using GeneScan version 3.1 software. Alleles sizes of the individual markers are determined by use of GenoTyper version 2.1 software.

To assess for linkage, we applied multi-point non-parametric linkage analysis using the MAPMAKER/SIBS software 2.0 . So far we identified 3 positive peaks on 3 different chromosomes. These peaks are currently further investigated with additional markers.

**Genome-wide scan in Attention Deficit Hyperactivity Disorder (ADHD).** *S.L. Smalley<sup>1</sup>, S.E. Fisher<sup>2</sup>, C. Francks<sup>2</sup>, A.J. Marlow<sup>2</sup>, I.L. MacPhie<sup>2</sup>, J.T. McCracken<sup>1</sup>, J.J. McGough<sup>1</sup>, L. Crawford<sup>1</sup>, T. Kim<sup>1</sup>, A. Liu<sup>1</sup>, S.F. Nelson<sup>1</sup>, A.P. Monaco<sup>2</sup>.* 1) UCLA Center for Neurobehavioral Genetics, Los Angeles, CA; 2) Wellcome Trust Centre for Human Genetics, Oxford, UK.

Attention deficit hyperactivity disorder (ADHD) is a common neurobehavioral condition affecting 5-10% of children and adolescents and 3% or more of adults. It is a condition characterized by symptoms of inattention and/or hyperactive-impulsive behaviors that result in impairment and have their onset in childhood. Genetic influences are predominant based on twin and family studies of ADHD, with estimates of heritability ranging from 60-80%. In an ongoing molecular genetic study of ADHD, we conducted a genome scan in 106 families including 128 Affected Sibling Pairs (ASPs). ADHD status was determined using a best estimate procedure with definite diagnosis following DSM-IV criteria and a probable diagnosis if a case fell one symptom short but met the impairment criteria. Sibs and their parents were genotyped with > 400 highly polymorphic markers spaced at 10cM intervals throughout the entire genome. Results from the scan revealed multipoint MLS values over 1 on chromosomes 10, 11, 12, 16, and 17 for ADHD (definite and probable). Restricting the analyses to those with only a Definite ADHD diagnosis led to MLS > 1 on chromosomes 4, 9, 10, 11, and 12. Additional genotyping in regions with MLS > 1 is in progress in an extended sample of ASPs. This is the first genome-wide scan to have been completed for this important neurobehavioural trait.

**A genome scan for quantitative trait loci influencing age of disease onset and central obesity in a UK population with Type 2 Diabetes.** *S. Wiltshire<sup>1,2</sup>, M. McCarthy<sup>1,2</sup> for the Warren 2 Consortium.* 1) Wellcome Trust Centre for Human Genetics, Oxford, UK; 2) Imperial College Genetics and Genomics Research Institute, London, UK.

Type 2 diabetes and central obesity contribute significantly to global mortality and morbidity. To investigate the genetic basis of central obesity in subjects with type 2 diabetes (T2D), together with factors affecting the progression of T2D, we have examined several quantitative traits from 573 sibships with T2D, from the Diabetes (UK) Warren 2 repository, for linkage to 418 autosomal microsatellite markers. Age of disease onset, body mass index (BMI), waist hip ratio (WHR), and waist and hip circumference measurements, adjusted for age and sex where appropriate, were analysed with Haseman-Elston (HE) and variance components (VC) methods, implemented in GENEHUNTER V2.0. Loci influencing age of onset were identified on 5p15 (HE-LOD=1.37; VC-LOD=1.17), 12p12 (HE-LOD = 1.92; VC-LOD=2.08, additive heritability = 32%), and 12q24 (HE-LOD=0.68; VC-LOD=1.87, additive heritability = 35%). The 12p locus coincides with linkage to T2D observed in US Europids with early onset T2D; that on 12q coincides with linkage to T2D in Finnish subjects from Botnia. There was evidence for linkage to BMI, waist and hip circumferences on chromosome 3q13 (HE-LOD scores of 1.34, 2.75 and 2.27); VC analysis yielded less significant evidence (LOD scores of 1.03, 1.86 and 1.81; additive heritabilities of 24%, 33% and 34%). This region is ~90cM centromeric to the linkages to BMI, waist and hip circumferences observed in the TOPS study and ~90cM from the linkage to BMI observed in the FUSION study. There was evidence for linkage to BMI, WHR, waist and hip circumferences on 12q21-24 (LOD score range: 0.59 (HE, waist) to 1.45 (VC, hip)), and to WHR, waist and hip circumferences on 19p13 (LOD score range: 0.86 (HE, hip) to 1.89 (VC, waist)). In summary, our study suggests the presence of one, perhaps two, loci on chromosome 12 modifying age of onset of T2D, a third locus on 3q influencing central obesity, and some evidence for loci on 12q and 19p influencing central obesity also.

Program Nr: 2073 from the 2001 ASHG Annual Meeting

**Weighting Pedigrees of Varying Sizes in Nonparametric Linkage Analysis.** *H. Sengul, D.E. Weeks, E. Feingolds.*  
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Over the last 10 years, allele-sharing statistics for "nonparametric" linkage analysis have become an important tool in mapping complex traits. Recent interest in applying these methods to larger and larger pedigrees has made it critical to understand how best to weight contributions from pedigrees of different sizes and configurations when combining them in a single study. For any given trait model and set of pedigrees, one can derive optimal weights, but such an exercise is of limited utility since one expects to have little knowledge of the trait model when studying a complex trait. We approach the problem by finding optimal weights for many trait models and pedigree structures and looking for weighting schemes whose power is robust over large classes of models. We then compare the power of our weighting schemes to that of the weighting schemes implemented in current software, including equal weighting of all families, weighting based on the number of affecteds, and weighting based on the square root of the number of affecteds.

**Test of association and estimation of heritability attributable to SNP effects for quantitative traits with ROMP.**

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With the availability of thousands of single-nucleotide polymorphisms (SNPs) in the human genome, testing for associations between functional SNPs and quantitative traits is becoming increasingly popular. Large scale implementation of these methods will require analytic methods that are computationally fast and that minimize the amount of genotyping. An extension of quantitative genetic theory based on the traditional linear regression of offspring on mid-parent method and the inclusion of a candidate locus effect as a covariate in the regression is presented. This method, ROMP, can be used to estimate the heritability of a quantitative trait, test whether the heritability of the trait is greater than 0, test for an effect of a SNP locus on that trait, and estimate the heritability attributable to that locus. The method uses linear regression and is most appropriately applied to parent-offspring trios or nuclear families, like the TDT. ROMP requires phenotyping data on both parents and offspring, but genotyping data only on the offspring. Unlike model-dependent methods, ROMP does not require the estimation of allele frequencies or genotype-specific means and variances or the computation of the proportion of alleles shared i.b.d. or i.b.s.

Computer simulation was used to determine the type I error rate and power of the method, and to determine the accuracy of estimates of the heritability of the trait and the heritability attributable to the candidate locus.

The ROMP method accurately estimated the heritability of the trait and the heritability attributable to a specific candidate locus. For the models considered, the type I error rate and power of ROMP were comparable to that of other methods. In addition, ROMP appears to be fairly robust with respect to the effects of population stratification. ROMP is computationally fast, does not require specialized software and can be implemented in standard statistical packages such as SAS.



**Genome screen to identify loci contributing to susceptibility for Parkinson Disease.** *N.D. Pankratz<sup>1</sup>, W.C. Nichols<sup>2</sup>, S.K. Uniacke<sup>2</sup>, A. Rudolph<sup>3</sup>, C. Halter<sup>1</sup>, E. Siemers<sup>4</sup>, J.P. Hubble<sup>5</sup>, P.M. Conneally<sup>1</sup>, T. Foroud<sup>1</sup> and Parkinson Study Group.* 1) Indiana University School of Medicine; 2) Children's Hospital Research Foundation; 3) University of Rochester; 4) Eli Lilly and Company; 5) Ohio State University.

Several genes have been identified which contribute to rare, early onset or autosomal dominant forms of Parkinson disease (PD). To identify susceptibility genes contributing to the more common, late onset form of PD, families consisting of at least one pair of living siblings diagnosed with PD were recruited through 58 Parkinson Study Group (PSG) sites distributed throughout North America. A Diagnostic Checklist was completed by the neurologist with inclusion criteria consisting of clinical features highly associated with autopsy-confirmed PD and exclusion criteria highly associated with other non-PD pathological diagnoses. Responses on the Diagnostic Checklist were then used to classify study subjects as having verified PD (230 subjects) or nonverified PD (78 subjects). A genome screen was completed using 382 markers. A total of 148 families having 2 or more affected siblings (162 affected sibpairs) were analyzed. Two models of affection status were considered. Model I included all individuals as affected, regardless of their final diagnostic classification, while Model II included only those individuals with a more stringent diagnosis of verified PD. We report the results of nonparametric linkage analyses of the sibpairs using MAPMAKER/SIBS, Aspex, and Allegro. The strongest finding across all methods was a lod score of 2.55 obtained on chromosome 13 with Model II. Other findings that were consistent across analysis methods include 6 regions with lod scores above 1.5: chromosomes 2 (lod=1.7, Model I), 4 (lod=1.8, Model I), 6 (lod=2.1, Model II), 11 (lod=1.6, Model II), 12 (lod=1.9, Model II), and X (lod=2.0, Model I). The regions on chromosomes 6 and 2 were previously implicated in genome-wide studies of PD and studies of the parkin gene (chromosome 6) are currently ongoing in the families demonstrating linkage to this locus. These results provide further evidence of the presence and location of genes that are involved in genetic susceptibility to PD.

**Refining the critical regions of 17q and 5p containing predisposing loci for multiple sclerosis using SNP-array and LD and haplotype sharing analysis.** *J.S. Saarela<sup>1,2</sup>, H.M. Frostad Riise Stensland<sup>1</sup>, D.C. Chen<sup>1</sup>, S. Finnilä<sup>1</sup>, A. Jokiaho<sup>1</sup>, A. Palotie<sup>3</sup>, L. Peltonen<sup>1,2</sup>.* 1) Department of Human Genetics, UCLA, School of Medicine, Los Angeles, CA; 2) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 3) Department of Pathology, UCLA, School of Medicine, Los Angeles, CA.

Multiple Sclerosis (MS) is one of the most common neurological diseases of young adults. The incidence (2-4/100000) and prevalence (50-100/100000) rates are highest in populations of Northern-European origin. We have used a study sample of 26 multiplex MS-families originating from Finland and established linkage to chromosomes 5p, 6p, 17q and 18q, all loci also identified in other populations. By using a population sub isolate and combined power of linkage and linkage disequilibrium (LD) as well as monitoring for shared haplotypes, the methods frequently used for the fine mapping of monogenic diseases, we have been able to restrict the critical regions for MS loci on chromosomes 17 and 5 from linked 23 and 20 cM intervals to 3 and 9 cM, respectively. The so far better-defined DNA-region on chromosome 17q contains a total of 144 predicted or confirmed genes, the corresponding number for chromosome 5p being over 170. We have selected SNPs in coding and regulatory regions of the predicted transcripts of the critical DNA regions and constructed an SNP-microarray, based on allele-specific primer extension. Out of 80 SNPs analysed so far, the majority show evidence for linkage, but thus far only one SNP mapping between the best multi-allelic markers on chromosome 17 reveals evidence for association in the Haplotype Relative Risk-test ( $p=0.001$ ). The SNP array is currently used to monitor for regional SNPs in a larger, population-wide study sample comprising of single affected MS-families.

**Genome scan linkage analysis of severe, early-onset COPD.** *E.K. Silverman<sup>1</sup>, J.D. Mosley<sup>1</sup>, M. Barth<sup>1</sup>, J. Senter<sup>1</sup>, A. Brown<sup>1</sup>, D.J. Kwiatkowski<sup>1</sup>, H.A. Chapman<sup>4</sup>, J.M. Drazen<sup>1</sup>, F.E. Speizer<sup>1</sup>, D.C. Rao<sup>2</sup>, M.A. Province<sup>2</sup>, E.J. Campbell<sup>3</sup>, S.T. Weiss<sup>1</sup>.* 1) Brigham & Women's Hospital, Boston, MA; 2) Washington University, St. Louis, MO; 3) University of Utah, Salt Lake City, UT; 4) University of California, San Francisco, CA.

Severe alpha 1-antitrypsin (AAT) deficiency is the only proven genetic risk factor for chronic obstructive pulmonary disease (COPD). To study novel genetic determinants of COPD, we enrolled 72 pedigrees ascertained through severe, early-onset COPD probands (FEV1 < 40% predicted, age < 53 years, without severe AAT deficiency). A 10 cM genome screen with short tandem repeat (STR) markers was performed by the NHLBI Mammalian Genotyping Service; 571 individuals were included in multipoint linkage analysis using ALLEGRO. Phenotypes analyzed included moderate airflow obstruction (FEV1 < 60% predicted, FEV1/FVC < 90% predicted), mild airflow obstruction (FEV1 < 80% predicted, FEV1/FVC < 90% predicted), and chronic bronchitis. Multipoint linkage analysis revealed the strongest evidence for linkage to the following locations: moderate airflow obstruction to chromosomes 12 (p=0.003) and 19 (p=0.004), mild airflow obstruction to chromosomes 8 (p=0.006) and 19 (p=0.01), and chronic bronchitis to chromosomes 19 (p=0.009) and 22 (p=0.006). Restricting linkage analysis to cigarette smokers provided increased evidence for linkage in several genomic regions, potentially related to gene-by-smoking interactions. Seven additional STR markers were genotyped on chromosome 12p; maximal evidence for linkage in all subjects was found to moderate airflow obstruction at 36 cM (p=0.0007) and to mild airflow obstruction at 38 cM (p=0.004). In sum, we have found multiple interesting regions with potential linkage to COPD-related phenotypes, with suggestive evidence for linkage of airflow obstruction to chromosome 12p. Further analysis of these chromosomal regions may lead to the identification of new genetic determinants of COPD. (Supported by NIH HL61575).

**Limits of resolution for genetic mapping of complex traits.** *G.J. te Meerman, I.M. Nolte.* Dept Medical Genetics, Univ Groningen, Groningen, Netherlands.

Success in finding mutant genes contributing to complex genetic disease has been minimal so far. This applies to finding approximate locations for contributing genes, by linkage and/or association analysis, as well as to the identification of responsible mutant genes, once association has been found. It seems likely that genotyping will become so cheap, that systematic whole genome screens, using thousands of individuals and tens of thousands of polymorphic markers, 1-3 per identified gene, will become practical in the near future. This will lead to the detection of genes involved in complex genetic disease, although the costs are several orders of magnitude higher than those required to identify rare and highly penetrant mutant genes by linkage analysis. The next question will then be: will we be able to pinpoint the genes and the mutations responsible for disease? The results until now do not give great reason for optimism: the association between markers and disease in the HLA area is strong, but responsible variants have not been identified e.g. in Multiple Sclerosis and Psoriasis. Frequent alleles that are functionally intact and have weak marginal effects cannot yet be identified from the genetic sequence alone. This underlines the necessity of knowing the limits of resolution of genetic mapping. We will show that on theoretical grounds the limit of resolution of statistical and logical gene mapping methods based on haplotype analysis (Beckmann et al, McPeck et al) may be suffice to identify causal polymorphisms, or to reduce the set of candidate causal variants to a few. Phase information is essential for this purpose and SNP based strategies present serious difficulties in this respect. We will demonstrate the asymptotic fine mapping behavior of haplotype sharing methods with some simulation examples. Beckmann L, Fisher C, Deck K-G, Nolte IM, Te Meerman G, Chang-Claude J. Exploring haplotypes sharing methods in general and isolated populations to detect gene(s) of a complex genetic trait. GAW-12 issue Genet Epidemiol 2001, in press McPeck MS, Strahs A. Assessment of linkage disequilibrium by the decay of haplotype sharing, with application to fine-scale genetic mapping. Am J Hum Genet. 1999 Sep;65(3):858-75.

**Evaluation of hybrid MCMC samplers for estimation of genes shared identical by descent and haplotyping.** *M. Szydlowski, S.C. Heath.* Dept of Epidemiology and Biostatistics, Mem Sloan Kettering Can Ctr, New York, NY.

Analysis of extended pedigrees and complex trait models is often beyond the power of exact algorithms. Monte Carlo techniques and the Gibbs sampler in particular have proven useful in many cases. In the presence of tightly linked loci, complex pedigree structure and large groups of full/halfsibs, however, simple samplers often suffer mixing problems ruining any inference. We evaluate the power of various hybrid samplers to tackle multilocus and close linkage problems on human pedigrees. Specifically, the ability of hybrid samplers to estimate the number of genes shared identical by descent (i.b.d.) and the probability distributions of haplotypes is evaluated. We used different pedigrees with both pure hierarchical and complex structures. The complex pedigree is from an isolated Micronesian Island population, consists of 253 individuals and contains several loops and large groups of fullsibs. A number of tightly linked codominant markers were simulated and analyzed with various hybrid samplers. The basic one was the whole locus sampler (L), which generates joint pedigree genotype configurations within a locus and ensures theoretical irreducibility of sampling chain. In addition to L updates we used three multilocus samplers: the whole meiosis sampler (M), which samples a joint segregation indicator pattern for all loci simultaneously and two simple Metropolis steps for sampling multilocus descent graphs in nuclear and three generation families in turn. In conclusion we demonstrate the great ability of hybrid samplers to perform efficient genetic analysis. The hybrid samplers present much better mixing properties when compared to simple samplers allowing for efficient estimates of i.b.d. sharing and haplotypes frequencies. Results on the application of different sampling schemes are presented and the generalization of the hybrid samplers for good performance across various pedigree structures is discussed. Some recommendations on the use of hybrid samplers are given. This work was supported by NIH Grant GM58757-01.

**An analysis of the influence of candidate genes on the refractive error of female DZ twins.** *Q. Prescott<sup>1</sup>, C.J. Hammond<sup>2</sup>, T. Andrew<sup>2</sup>, A.M. Dearlove<sup>3</sup>, T.D. Spector<sup>2</sup>, S.S. Bhattacharya<sup>1</sup>, A.R. Webster<sup>1</sup>.* 1) Dept of Molecular Genetics, Institute of Ophthalmology, London; 2) Twin Research and Genetic Epidemiology Unit, St Thomas Hospital, London; 3) HGMP Resource Centre, Hinxton, Cambridge, UK.

The development of refractive error in humans is due to an aberration of the process of emmetropization, in which the eyes' refractive components develop interdependently to form an accurately focused image on the retina of a distant object of regard. The susceptibility to such aberration is likely due to both environmental and genetic effects. We present a study to determine the contribution of candidate genes in refractive error development in an all-female cohort of dizygotic (DZ) twin pairs (age range 49-79 years, mean 62.4 years) from the St Thomas twin study, recruited independently of ophthalmic status or history. The spherical equivalent and astigmatic cylinder was measured on all subjects (mean +0.8D, range -7.5D to +10.8D). So far alleles of intra- and juxta- genic polymorphisms in two candidate genes have been scored: fibrillin1 and collagen 11a1, mutations in which cause Marfan and Stickler syndrome respectively. Both these disorders are consistently associated with moderate to high myopia in affected individuals. Using refractive error as a quantitative trait, this resource allows the assessment of the influence of these and other candidate genes in the determination of refractive status by the analysis of both allele-sharing and association of refractive status with specific alleles and haplotypes.

**Genetic Susceptibility to Juvenile Rheumatoid Arthritis.** *J.A. Runstadler<sup>1</sup>, K. Laiho<sup>2</sup>, J. Tuomilehto<sup>2</sup>, M.F. Seldin<sup>1</sup>.*  
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This study was undertaken to further define the genetic susceptibility to Juvenile Rheumatoid Arthritis (JRA) using Finnish JRA probands, family and population controls. HLA allele associations have often been weak in JRA studies and dependent on the population being examined. Linkage may therefore be implied where none exists, due to population stratification. In addition, the involvement of other genes within the HLA region has not been ruled out. The current study of JRA utilizes a population (Finnish) with the potential advantage of genetic isolation. In this population, problems due to population stratification and locus heterogeneity are reduced, allowing for greater power. This study also utilized the transmission disequilibrium test (TDT) in addition to a case/control design to examine JRA and JRA subtypes. We have examined 21 microsatellites and 2 insertion/deletion markers spanning an 18 cM region of chromosome 6 encompassing the entire MHC. Each marker was genotyped for a set of 301 Finnish JRA nuclear families and 500 Finnish control individuals. Analysis by association and TDT methods each demonstrated strong evidence for a susceptibility locus near the DQCAR microsatellite ( $p < 0.000001$  for both methods) which is flanked by DQB1 and DRB1. Analysis of closely linked microsatellite markers to DRB1 suggest that DRB1\*0801 and DRB1\*1101 rather than DQA1 or other HLA alleles may be responsible for conferring susceptibility to disease while DRB1\*0401 is protective. These findings are consistent with the most compelling results of previous reports on HLA associations. Subdividing the data by Pauciarticular and Polyarticular RF- subtypes of JRA did not alter the strong association at the DQCAR locus ( $p < 0.000001$ ). Modeling for gene action using TDT data shows the strongest evidence exists for an additive or dominant gene effect at this locus. In Pauciarticular patients, the strong association does not extend proximal to markers MID-104 and MID-108 as it does in Polyarticular patients ( $p < 0.00001$ ). In addition, two other loci within the 18 cM region we examined show association ( $p < 0.05$ ) but are highly dependent on the subclass of JRA examined.

**Identification and genetic analysis of polymorphic loci in the human Nurr1 transcription factor gene NR4A2.**

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NR4A2, or Nurr1, is an orphan nuclear receptor implicated in the development of dopaminergic cells of the ventral midbrain. Mice lacking Nurr1 fail to develop dopaminergic cells of the ventral tegmental area (VTA) and the substantia nigra (SN). The dopaminergic cells of the VTA provide innervation to the prefrontal cortex, a signaling system believed to be of major importance to the pathogenesis of neuropsychiatric disorders such as Schizophrenia and ADHD, suggesting that Nurr1, or one of its targets is a potential candidate gene for these disorders. Identification of mutations or polymorphisms in genes that control the development and/or function of the dopaminergic signaling pathways, such as Nurr1 is an important primary step in candidate gene studies for these disorders. We have identified several polymorphic loci in the Nurr1 gene via database searches of the high throughput sequencing database as well as an SSCP screen of Nurr1 coding regions. Database analysis revealed a CA microsatellite repeat polymorphism in the 3' UTR of this gene which was confirmed by PCR. Two alleles were identified, differing by one repeat length. These alleles were tested for an association with ADHD in both a case control study of individuals from the Milwaukee Longitudinal Study of ADHD, and in 35 sib pairs with ADHD and their parents. A trend ( $.25 > p > .1$ ) towards increased transmission of the shorter allele (allele 1) was observed in families with ADHD via TDT analysis. SSCP screening in 90 individuals revealed a rare polymorphism in intron 5 present in one individual, and a more common polymorphism -263 DC in the promoter region with a frequency of approximately .30. Ongoing studies will examine the association of this promoter polymorphism to ADHD as well as determine its effects on the transcriptional regulation of Nurr1.



**Evidence of linkage for speech impairment with chromosome 7 markers.** *J.H. Schick<sup>1</sup>, A.M. Kundtz<sup>1</sup>, H.G. Taylor<sup>2</sup>, L.D. Shriberg<sup>3</sup>, L.A. Freebairn<sup>2</sup>, B.A. Lewis<sup>2</sup>, S.K. Iyengar<sup>1</sup>*. 1) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 2) Department of Pediatrics, Rainbow Babies and Childrens Hospital, Case Western Reserve University, Cleveland, OH; 3) Waisman Center on Mental Retardation and Human Development, University of Wisconsin-Madison, Madison, WI.

Speech and language are important human characteristics. The etiology of speech and language impairment is largely unknown, but family and twin studies have indicated a significant genetic component. An autosomal dominant locus (SPCH1) for a severe speech and language disorder was localized to a 5.6 cM region of chromosome 7q31 between D7S2459 and D7S643 (Lai et al., 2000) in a large three-generation pedigree characterized with dyspraxia of speech. We tested the hypothesis that a candidate locus on 7q31 also segregated with a broader phenotype of speech and/or language disorders in nuclear families. We analyzed 7 markers (D7S1789, D7S527, D7S1812, D7S821, D7S2425, D7S692, & D7S486) spanning a 21.26 cM region on 7q31 in a sample ascertained through pre-school probands. Eighty-nine families (N=427) consisting of 196 sib pairs were genotyped. SIBPAL2 (S.A.G.E., Beta 7) was used for single- and multi-point linkage analysis for two binary traits: speech impairment and speech or language disorders. For each binary trait, the linkage analysis was adjusted for: age and socio-economic status. Our preliminary multipoint analyses suggest that a putative gene for the speech only phenotype is located near a 0.55 cM region containing markers D7S527 ( $p=0.0009$ ), D7S1812 ( $p=0.0007$ ) and D7S821 ( $p=0.0062$ ) and flanked by markers D7S1789 ( $p=0.1456$ ) and D7S2425 ( $p=0.0997$ ) located 4.96 cM and 8.60 cM away, respectively. No locus for the broader phenotype of speech and/or language disorders appears to segregate in these families. This study is the first to provide support for the earlier hypothesis that a putative gene for speech disorders localizes to 7q31. Haplotype-based analyses are being conducted and are an initial step in the isolation of the first gene for speech disorders. This research is supported by NIH grants NIDCD-00528 and NIDCD-04005-01.

**Quasi-linkage: a confounding factor in linkage analysis of complex diseases.** *S. Sivagnanasundaram*<sup>1</sup>, *K.W. Broman*<sup>2</sup>, *A. Paterson*<sup>3</sup>, *A. Petronis*<sup>1</sup>. 1) Neurogenetics Section, Centre for Addiction and Mental health, Toronto, CA; 2) Dept of Biostatistics, The Johns Hopkins University, Baltimore, MD; 3) Dept of Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, CA.

According to Mendel's second law, unlinked genomic loci segregate independently during meiosis, and this directly applies to non-homologous chromosomes. An exception to this rule is the phenomenon of quasi-linkage (QL) that describes the non-random segregation of non-homologous chromosomes. Molecular mechanisms of QL are not clear, and so far QL has been explained by the 'affinity hypothesis', that is the tendency of non-homologous chromosomes, through the mutual attraction of their centromeres, to migrate together. QL was initially observed in mice and plants but has not been investigated in humans. We were interested in determining if QL served as a significant confounding factor, that is quasi-linked loci falsely exhibiting co-segregation with disease, in genome scans for complex diseases. We investigated individual markers showing linkage to schizophrenia, asthma, multiple sclerosis, inflammatory bowel disease and type 1 diabetes. 'Disease linked' markers were tested for QL in a pairwise linkage analysis against all other markers exhibiting evidence for linkage in each specific disease. Marshfield genotyping dataset of eight CEPH families was used for this purpose. A wide range of QL maximal lod scores were observed. The highest lod score of 2.28 (theta F= 0.4; theta M= 0.32) was generated for two markers on chromosomes 15p13-q12 and 9q32-q34 which initially demonstrated evidence for linkage to schizophrenia (Kaufmann et al. 1998). A large number of QL lod scores varied between 1 and 2, and although did not reach the level of statistically significant linkage, are within the range of the overwhelming majority of "lukewarm" loci of complex diseases. The above data suggest that QL may be an important confounding factor in the genetics of complex traits, and if so should be taken into account when oligogenic and polygenic traits are investigated. Genome-wide QL maps of reference families would aid in accounting for such confounding effect.

**GenoMapper; mapping system of mouse full length cDNA onto genomic sequences.** *K. Sakai<sup>1</sup>, H. Kiyosawa<sup>1,2</sup>, S. Kondo<sup>1</sup>, I. Yamanaka<sup>1</sup>, T. Saito<sup>1</sup>, Y. Hayashizaki<sup>1,2,3</sup>.* 1) Genome Exploration Res Grp, RIKEN, Genomic Sci Ctr, Yokohama, Japan; 2) Division of Genomic Science, Yokohama City University, Yokohama, Japan; 3) Department of Medicine, Tsukuba University, Tsukuba, Japan.

We developed mapping viewer system to facilitate identifying the candidate genes for positional candidate cloning. In order to construct mouse gene encyclopedia, we have been producing tens of cDNA libraries, isolating cDNA clones, sequencing, and mapping them onto the human genome draft sequences. Our group has also been studying biological profiles of the cDNAs, such as expression analysis by DNA microarray technique and protein-protein interaction (PPI) analysis by mammalian two hybrid assay. The mapping method based on blast was described in our previous paper (Kondo et al.). We mapped mouse cDNAs, along with human RefSeq and the representative sequences of UniGene clusters, onto the human genome draft sequences assembled at University of California, Santa Cruz. The exon predictor programs (GenScan and FgenesH) were also used to identify the possible coding region. Our cDNAs were classified according to their novelty; 1) there are also known genes in the same position on the chromosomes, 2) there are also ESTs, 3) there are only predicted exons by the computer programs, and 4) there are no other genes at all. The description page for each cDNA contains its chromosomal position with links to other data such as alignment to genome sequences, expression, PPI, ORF analysis, FANTOM annotation page (The RIKEN Genome Exploration Research Group Phase II Team and FANTOM Consortium) and also to the external database such as OMIM and Locus Link. Position search is provided to define the cDNAs mapped to the region of interest. All the data gathered and linked to GenoMapper are utilized to narrow down the candidate genes for the phenotypes of interest. The strength of GenoMapper is that it contains novel genes only we have and biological data to reduce the numbers of candidate genes. Overview of GenoMapper system will be presented. Kondo et al. *Mamm. Genome*, in press. The RIKEN Genome Exploration Research Group Phase II Team and FANTOM Consortium, *Nature* 409, 685-690. 2000.

**Homozygosity mapping of three idiopathic mental retardation loci : toward identification of the first autosomal recessive isolated mental retardation gene.** *L. Colleaux<sup>1</sup>, F. Molinari<sup>1</sup>, M. Rio<sup>1</sup>, A. Rothchild<sup>2</sup>, A. Migharbane<sup>3</sup>, A. Munnich<sup>1</sup>.* 1) INSERM U393, Hosp Necker-Enfants Malades, Paris, France; 2) Hadassah Medical school, Jerusalem, Israel; 3) Universite Saint Joseph, Faculte de Medecine, Beyrouth, Liban.

Mental retardation (MR) is the most common developmental disability, affecting largely 2% of the general population. The causes of MR are diverse, but an autosomal recessive mode of inheritance may account for a significant proportion of mentally retarded individuals. The extreme genetic heterogeneity of idiopathic MR and the unavailability of large family pedigrees of nonsyndromic autosomal recessive MR has limited the use of genetic linkage to identify the disease causing genes. While a large number of X-linked mental retardation genes have been found, none of the numerous genes involved in autosomal recessive MR have been hitherto identified. Here we report on the homozygosity mapping of the disease gene in three unrelated inbred families. Genome-wide homozygosity mapping was performed using microsatellite markers at an average distance of 10cM. For each family, the results provided evidence of homozygosity for a single region on chromosomes 11p15.5, 2q13 and 4q24 respectively. Our results emphasize the difficulty encountered in some families to reduce the homozygosity region to a small workable region. However, in one of our family, fine genetic mapping and study of unaffected members of the family allowed us to map the disease causing gene to a small region of about 1.5 Mb.

**GENOME-WIDE SEARCH FOR TYPE2 DIABETES SUSCEPTIBILITY GENES IN JAPANESE AFFECTED SIBPAIRS.** *P. Froguel*<sup>1,3</sup>, *Y. Mori*<sup>1</sup>, *S. Otabe*<sup>1</sup>, *C. Dina*<sup>1</sup>, *K. Yasuda*<sup>2</sup>, *C. Lecoeur*<sup>1</sup>, *K. Hara*<sup>2</sup>, *K. Tobe*<sup>2</sup>, *P. Boutin*<sup>1</sup>, *T. Kadowaki*<sup>2</sup>. 1) Institute of Biology - CNRS UPR-ESA 8090, Lille Cedex, France; 2) Department of Metabolic Diseases, University of Tokyo, Tokyo, Japan; 3) Barts and the London Genome Centre, London, UK.

**Background and Aims :** The genetic background which predisposes to type2 diabetes in the Japanese population is largely unknown. To search for major susceptibility loci, we conducted a 10-cM genome scan for type 2 diabetes traits. **Materials and Methods :** A total of 359 individuals of Japanese origin were ascertained in Japan from 159 families, including 229 affected sibpairs. In 202 males, mean age at diagnosis was 45.9±9.7 yrs and maximal BMI was 26.4±3.0 kg.m<sup>-2</sup>. In 157 females, mean age at diagnosis was 48.3±11.1 yrs and maximal BMI was 27.4±3.8 kg.m<sup>-2</sup>. Fluorescent microsatellite marker set (ABI Linkage Mapping Set version2, MD10) was used for the primary 10cM scan. Additional 33 markers were typed for 13 candidate genes, mainly transcription factors. Non-parametric multipoint linkage analyses were performed by MLBGH1.0, MAPMAKER/SIB2.0 and GENEHUNTERPLUS. **Results:** Non-parametric multipoint linkage analyses performed in the whole population showed 7 potentially linked regions, on 1p36-p32, 3q26-q27, 6p23, 7p22-p21, 11p14-p13 (near Pax6), 15q13-q15 and 20q12-q13 (near HNF4 alpha). Subset analyses according to maximal body-mass index and to early age at diagnosis showed suggestive evidence of linkage with type 2 diabetes on 7p22-p21 (a maximum lod=4.46 in 85 families that were increasingly-ordered-ranked by maximal BMI), 15q13-q15 (MLS = 3.62) and 20q12-q13 (MLS = 2.56). **Conclusions :** These results confirm previous indication for linkage found on chromosome 3q, 15q and 20q and identifies new potential locus on 7p that may confer genetic risk for type 2 diabetes in the Japanese population.

**Single nucleotide polymorphism genotyping in DNA pools.** *E.L. Meaburn, R. Plomin, L. Schalkwyk.* Social, Genetic & Developmental Psychiatry Research Centre, Institute of Psychiatry, Kings College London, De Crespigny Park, London SE5 8AF, UK.

The advent of publicly available single nucleotide polymorphism (SNP) databases has made it increasingly feasible to construct dense marker maps of SNPs for use in allelic association studies of complex diseases. However, a persistent problem is the vast amount of genotyping that is required for such studies, especially for the large samples needed to detect associations of small effect size. DNA pooling facilitates genotyping of large samples because DNA is pooled from all individuals within each phenotypic group. Each pool, rather than each individual is genotyped and the relative allele frequencies of each group are compared. However, DNA pooling has only been used with fluorescent tags on short-sequence repeat markers that detect length polymorphisms, whereas it would be advantageous to use SNPs in DNA pooling research. For this reason we evaluated three SNP genotyping methodologies for their accuracy, robustness, and efficiency for DNA pooling using ten publicly available SNPs in coding regions. We used 1) competitive allele specific fluorescent probes in the Taqman (5' nuclease) assay, 2) Kinetic PCR using allele specific primers and SYBR green (Genome research 2000 10;258-266) and 3) the SNaPshot ddNTP Primer extension reaction kit. We conclude that all three SNP genotyping methods can be extended to DNA pooling. All three methods discriminate over a wide range of allele frequencies and yield accurate pooling results as confirmed by individual genotyping. However, as the polymerase chain reaction is a critical step in all the reactions, we conclude that methods employing real time quantification are more robust than methods that employ endpoint analyses.

Program Nr: 2089 from the 2001 ASHG Annual Meeting

**Analysis of short tandem repeats D3S1358, TH01, TPOX and CSF1P0 with an infrared fluorescent automated DNA sequencer (LI-COR 4200) using an alternative PCR strategy.** *U. Ricci, I. Sani, M.L. Giovannucci Uzielli.* Genetics and Molecular Medicine University of Florence Department of Paediatrics, Azienda Ospedaliera A.Meyer (Italy).

Recently we demonstrated the possibility to analyze a battery of thirteen STRs loci TPOX, D3S1358, FGA, CSF1PO, D5S818, D7S820, D8S1179, TH01, vWA, D13S317, D16S359, D18S51, D21S11 and the X-Y homologous genes amelogenin system by using an infrared-based fluorescent automated DNA sequencer (Electrophoresis 2000;21:3564-3570). We used these systems for paternity testing, UPD-studies and also linkage analyses. No doubt that the analyzed loci are of particular interest for the forensic community, because they represent the core of the Combined DNA Index System (CODIS), used from FBI. Here we introduced a new PCR strategy we used to analyze the multiplex system D3S1358, TH01, TPOX and CSF1PO (called MU2). The previously reported primers for TH01 were substituted with the primers suggested from Edwards et al. (1991). The amplification products were generated using Forward and Reverse unlabelled primers with a little portion of labelled Forward primer, covalently linked at the 5' end to an IR-fluorescent dye (IRDye800). The test resulted no expensive, robust, efficient and extremely sensible. In fact we were able to detect less of 10 pg of genomic degraded DNA. Here we report our results in analyze of bloodstains, archival formalin-fixed paraffin-embedded tissue and six months old histologic sections. Moreover, our test was compared with a capillary fluorescent automated sequencer and the results were in complete agreement, confirming the comparability of the IR-technology with the UV-technology.

**Loci mapping to chromosomes 3 and 9 affect left ventricular dilatation and survival in a transgenic mouse model of dilated cardiomyopathy and heart failure.** *K.M. Carlson<sup>1</sup>, M. Suzuki<sup>2</sup>, N. Golson<sup>2</sup>, H.A. Rockman<sup>2</sup>, D.A. Marchuk<sup>2</sup>.* 1) Genetics, Duke University, Durham, NC; 2) Cardiology, Duke University, Durham, NC.

Cardiac specific over-expression of calsequestrin (CSQ), a sarcoplasmic reticulum  $\text{Ca}^{2+}$  binding protein, in transgenic mice results in cardiac hypertrophy followed by the development of dilated cardiomyopathy (DCM) and premature death. Genetic background plays an important role in the manifestation of DCM in this model. In a DBA/2J (DBA) background, over-expression of CSQ results in depressed cardiac function by 14 weeks of age and premature death by 20 weeks of age. In contrast, the F1 progeny from a DBA-CSQ cross to C57BL/6 (B6) exhibit greater ventricular dilatation and depressed cardiac function by 4 weeks of age and death by 8 weeks of age. In order to map modifier genes acting in this model, we backcrossed DBA/B6 F1-CSQ animals to the parental DBA strain and performed a genome wide screen of 64 N2 animals. We performed quantitative trait loci (QTL) mapping to identify QTLs linked to two phenotypic measures: (1) left ventricular end diastolic diameter (LVEDD) and (2) survival. Using single locus association tests, a single locus on chromosome 3 was significantly linked to survival with a LRS of 24.1 ( $p < .001$ ). Furthermore, the same interval on chromosome 3 was significantly linked to LVEDD with a LRS of 24.3 ( $p < .001$ ). These QTLs were confirmed by performing simple interval mapping using additional progeny and markers. The maximum LRS reached was  $\text{LRS} = 26.5$  ( $p < .001$ ) and  $\text{LRS} = 32.1$  ( $p < .001$ ) for survival and LVEDD respectively. Because survival and ventricular dilatation are correlated in this model, these two loci probably represent the same locus. Using composite interval mapping to mask the effect of the chromosome 3 locus, an additional QTL linked to survival was mapped on chromosome 9 with a LRS of 14.0 ( $p < .05$ ). Our data indicate that multiple genetic modifiers including a strong locus on chromosome 3 and a weaker locus on chromosome 9 are working together to influence cardiac function and survival in the CSQ model of DCM. The identification of these modifiers will enable us to study their role in modifying the outcome of DCM in human patients.



**X-linked myopathy with excessive autophagy (XMEA): clinical spectrum, refined mapping and mutation screening.** *S.A. Alic<sup>1</sup>, R. Aiyar<sup>1</sup>, V. Juel<sup>2</sup>, J. Mandell<sup>2</sup>, M. Villanova<sup>3</sup>, M. Fardeau<sup>4</sup>, S.W. Scherer<sup>1</sup>, M. Auranen<sup>5</sup>, H. Kalimo<sup>6</sup>, B.A. Minassian<sup>1</sup>.* 1) Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 2) U. Virginia; 3) Inst. Orthopedics Rizzoli, Bologna, Italy; 4) INSERM U533, Paris, France; 5) Nat. Pub. Health Inst., Helsinki, Finland; 6) U. Turku, Finland.

Among the inherited vacuolated myopathies two are X-linked (but not allelic) and both are characterized by lysosomal pathology. One, Danon disease, is associated with cardiomyopathy and mental retardation in addition to skeletal myopathy, and the other, XMEA, is not. In both conditions, vacuoles contain cellular debris. The defective protein in Danon disease has recently been identified as LAMP2, a lysosome associated membrane protein. LAMP2 appears necessary for the maturation of vacuoles into phagosomes following fusion with lysosomes (Nature 2000;406,902-910). The XMEA gene is unknown. We and others recently mapped this gene to the last 7Mb of the long arm of the X chromosome distal to DXS1193. In this communication we describe new XMEA families and show that the disease can present either in infancy/early childhood or in late teenage/early adulthood and that both forms link to the same locus and are therefore likely allelic. We also present identification of a founder haplotype in French families which suggests a narrowing of the gene locus by 2Mb to telomeric to DXS8103. Finally, we report the first American XMEA family and identify a recombination event in this family which confirms the centromeric boundary at DXS8103. Screening of functional candidate genes from the remaining 5Mb for mutations is now underway with 7 genes sequenced to date.

**A Novel Distal Myopathy with Rimmed Vacuoles in a Large Saudi Kindred is Linked to Chromosome 12.** V.A. Bharucha, E.J. Cupler, S.A. Bohlega, C.E. Boumah, S.N. Abu-Amero, M. Kambouris, B.F. Meyer, C.A. Hodgkinson. Biological & Medical Research, KFSH&RC, RIYADH, SAUDI ARABIA.

DMRV is an autosomal recessive myopathy that preferentially involves the anterior compartment lower extremity muscles. Histopathological features reveal rimmed vacuoles with filamentous cytoplasmic and nuclear inclusions (Nonaka, 1981). Hereditary inclusion body myositis (HIBM) and DMRV share some clinical and histological features. These 2 disorders map to overlapping regions of chromosome 9 suggesting that they may be allelic (Ikeuchi, et al. 1997). Their analysis revealed that the DMRV gene is located within a 23.3cM interval defined by D9S319 and D9S276 on chromosome 9. We have analyzed a large inbred kindred from Saudi Arabia in which 12 individuals are affected with distal myopathy, wherein rimmed vacuoles are observed in muscle biopsy. The disorder appears to be transmitted as an autosomal recessive trait. Affected and unaffected individuals were genotyped using markers surrounding and within the region of chromosome 9 previously reported to link to DMRV and HIBM. No linkage was found to this region of chromosome 9. Using markers D2S21, D2S13, D2S291 and D2S21 linkage to the dysferlin gene was also excluded. These data, therefore indicate that this disorder represents a novel clinical and genetic entity. A whole genome scan of affected and unaffected individuals revealed linkage to chromosome 12q with a multi-point LOD score of 3.3 based on an autosomal recessive pattern of inheritance. Additional family members are being analyzed to further refine the interval. These analyses exclude both disorders previously described with clinical similarities to this distal myopathy.

**Allele-specific amplification utilizing novel fluorescent primers for the detection of single nucleotide polymorphisms.** *M. Darfler, A. Gallego, I. Nazarenko, J. Solus.* Molecular Biology R&D, Invitrogen, Rockville, MD.

We have developed assays for detection of single nucleotide differences utilizing homogeneous phase fluorescence detection methods. These assays are based on the use of primers labeled with a single fluorescent dye which do not require a specific quenching moiety. These fluorescent primers exhibit a large increase in intensity when incorporated into double stranded DNA. This property provides a means for real-time or endpoint detection of nucleic acids in a closed tube format that requires no additional steps subsequent to the PCR.

Demonstrated are the use of these fluorescent primers in allele specific amplification (ASA) reactions for both real-time detection during PCR or detection after PCR. Enhancement of the allele discrimination is shown with improvements in the design of the allele specific primer and a unique DNA polymerase which helps to increase the specificity of the allele specific PCR amplification. The combination of these improvements along with a flexible homogenous phase detection system provides a simple and reliable technique for genotyping SNP targets.

The allele specific amplification reaction has also been adapted to a universal allele detection design, where the 5'-end of the ASA primer contains a tail sequence which is identical to the 3' portion of a labeled universal detection primer. This detection scheme eliminates the expense of designing sequence specific detection primers for each specific target in the reaction, since it allows the utilization of the same detection primer for multiple targets. The detection primer design is compatible with alternative fluorescent labels providing the capability of biallelic genotyping reactions. We demonstrate duplex reactions using two universal allele detection primers labeled with FAM and JOE dyes for real-time detection or with FAM and TAMRA dyes for endpoint analysis. Multiple platforms are shown for the analysis of the ASA reactions: real-time detection instruments, fluorescence plate readers and automated fluorescence sequencers for higher throughput of multiple SNPs by separation of several targets of different sizes in a multiplex PCR.

**Mutation screening of the SynGAP gene in patients with dyslexia.** *K.E. Deffenbacher<sup>1</sup>, D.M. Hoover<sup>1</sup>, J.B. Kenyon<sup>1</sup>, R.K. Olson<sup>2</sup>, J.C. DeFries<sup>2</sup>, S.D. Smith<sup>1</sup>*. 1) Dept Molec Genetics, Univ Nebraska Med Ctr, Omaha, NE; 2) Inst Behav Genetics, Univ Colorado, Boulder, CO.

A quantitative trait locus for dyslexia has been localized to a 10cM region on 6p21.3. A 9.5 Mb contig spanning this region has been assembled and was examined for possible candidate genes. SynGAP, a synaptic RasGAP, mapped to this region and was selected as a candidate. SynGAP is highly enriched at excitatory synapses and is associated with NMDA receptors within the postsynaptic density. NMDA receptors play a pivotal role in synaptic plasticity which is, in part, transcription dependent and underlies processes such as long term potentiation, learning and memory. NMDA signaling to the nucleus can occur through activation of the Ras/MAPK pathway which, in turn, is negatively regulated by the GTPase stimulating activity of SynGAP. Based on this putative role in modulating synaptic plasticity SynGAP was selected as a candidate gene for dyslexia. SynGAP is an 1135 amino acid protein that was previously cloned and characterized. Exons 1 to 16 had been predicted and annotated on clone AL050332. Blast search of the 3' end of this clone identified an overlapping clone (AL161903) on which the remaining 3 exons (17-19) were characterized by manual exon prediction. Intronic primers were designed flanking the 19 exons for analysis of the coding regions and splice sites. Mutation screening was performed by direct sequencing using a subset of 47 dyslexic children. Subjects in this subset were selected for a discriminant score and/or nonword recognition phenotype of  $\pm 2$  standard deviations since these phenotypes show strong linkage on 6p21.3. Novel polymorphisms were detected and will be sequenced in 96 control samples for comparison.

**Inherited point mutations in the TPMT gene: CDCE/hifi PCR scan of 37,000 alleles.** X. Li-Sucholeiki, B. Glassner, T. Thompson, A. Tomita-Mitchell, B. Karger, W. Thilly. Peoples Genetics, Woburn, MA.

We have developed a scanning technology employing constant denaturant capillary electrophoresis (CDCE) coupled with high-fidelity (hifi) PCR for point mutations occurring at frequencies as low as  $5 \times 10^{-5}$ . This technology permits the discovery of inherited point mutations in large pooled human samples. As an example, we studied the thiopurine S-methyltransferase (TPMT) gene in 18,707 juveniles. TPMT catalyzes the S-methylation of thiopurine drugs used in cancer treatment. Genetic polymorphism in TPMT has been associated with individual variations in thiopurine toxicity and efficacy. We scanned the coding regions and splice sites, and identified and determined the frequencies of a set of point mutations with frequencies ranging from 14% to 0.01% in this population. The set of mutations observed included a series of novel mutations in addition to those previously reported. Some of the novel mutations may be of clinical importance for thiopurine therapy. This study demonstrates the power of CDCE/hifi PCR as a tool for the comprehensive discovery of mutations in large populations.

**Detection of Sequence Variations in CYP2D6 in Human Diversity Panel DNAs by a New Method for Genetic Variation Screening.** *E.P. Dawson<sup>1</sup>, C.D. Cooper<sup>1</sup>, J.Q. Steward<sup>1</sup>, J.A. Phillips,III<sup>2</sup>*. 1) BioVentures,Inc, Murfreesboro, TN; 2) Vanderbilt University School of Medicine, Nashville, TN.

Genetic variations have been described for a number of drug-metabolizing enzymes, in particular the cytochrome p450s. Some of these variations contribute to the differences in the efficacy or toxicity of many pharmaceutical medications that are observed between individuals. CYP2D6 is an hepatic cytochrome p450 which is involved in the detoxification or activation of a number of important pharmaceuticals and is also thought to play a role in the activation of certain xenobiotics to carcinogens. CYP2D6 has over 70 reported allelic variants. We determined the extent of CYP2D6 sequence variation in defined human diversity panels to establish the frequencies of the reported variants, as well as any unreported variants which might alter the function of CYP2D6. To do this, we utilized genetic variation screening (GVS) to interrogate all 9 exons of CYP2D6 in genomic DNA's of the diversity panels for nucleotide variations. DNA's from over 200 individuals representing 400 alleles were analyzed and variations found by GVS were confirmed by Sanger sequencing. We found several of the previously described CYP2D6 variants and identified multiple variants not previously described in DNAs from the diversity panels. Surprisingly, the frequencies of some variants were different than expected from previous reports in the literature. Our results indicate that: 1) CYP2D6 variation occurs more commonly than expected in defined human diversity panels and 2) the extent and character of this variation can be determined by GVS.

**In Search of Functional Polymorphisms in the *MDR1* Multidrug Transporter in the Asian Population.** *K. Tang*<sup>1</sup>, *S.L. Kao*<sup>1</sup>, *I. Cheong*<sup>1</sup>, *H.C. Liew*<sup>1</sup>, *S.M. Ngoi*<sup>1</sup>, *C.G.L. Lee*<sup>1,2</sup>. 1) Department of Biochemistry, National University of Singapore, Singapore; 2) Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, USA.

Success in long-term cancer chemotherapy is limited by the acquired resistance of cancer cells to diverse structurally-different anti-cancer drugs. One of the major contributors to this acquired resistance is the overexpression of the multidrug resistance (*MDR1*) gene. The *MDR1* gene encodes a 170 kDa P-glycoprotein and belongs to the ATP-binding cassette (ABC) family of transporters. It actively effluxes diverse, structurally unrelated compounds and drugs including several non-cancer drugs. The tissue distribution of the *MDR1* transporter at the interface of important organs (e.g. blood-brain barrier) suggests that this transporter plays an important role in determining the bioavailability of drugs as well as concentration of drugs in sensitive organs like the brain and the fetus. Various studies have suggested that the substrate binding sites within *MDR1* lie in the transmembrane regions 5, 6, 11 and 12. Mutations within the substrate binding regions and elsewhere were found to change substrate specificity of the *MDR1* transporter. Recently, several polymorphisms in the *MDR1* gene in the Caucasian population have been identified. As there are differences in drug response amongst different ethnic groups, we would like to examine the frequency of reported *MDR1* polymorphisms as well as identify novel polymorphisms in our population, which comprises Chinese, Malays, Indians and Eurasians, mainly of Asian origin. Using allele-specific restriction enzyme site analysis, we did not find the reported polymorphism at position 61 of exon 2 (A@G) in 100 individuals studied. However, we identified a putative novel polymorphism in the promoter region of the *MDR1* gene and are in the process of confirming and testing its functionality in a robust reporter assay that we developed. We are also screening for other novel polymorphisms within the *MDR1* gene prevalent in our population and correlating these polymorphisms with *MDR1* gene expression and drug transport using a cell-based drug transport assay.

**A Comparison of EM and Gibbs sampling methodologies in estimating population haplotype frequencies and individual haplotypes in a phase-unknown situation.** *A.B. Seymour<sup>1</sup>, D.M. Meyer<sup>2</sup>, G.R. Warnes<sup>2</sup>, D.P. Dean<sup>3</sup>, S.M. Webb<sup>1</sup>.* 1) Pharmacogenomics, Pfizer, Groton, CT; 2) Biostatistical Reporting, Pfizer, Groton, CT; 3) Discovery Informatics, Pfizer, Groton, CT.

Pharmacogenomic investigation into the role candidate genes play in determining response to certain therapies and to disease susceptibility has been greatly facilitated with the discovery and mapping of novel single nucleotide polymorphisms as a by-product of the human genome sequence completion. Understanding the segregation of SNPs across a candidate gene as a haplotype can increase the information content within each gene and provide an increased likelihood of identifying a locus that contributes to phenotypic variation. Quantitating these haplotypes within a population of unrelated individuals is necessary for testing pharmacogenomic hypotheses. Current high-throughput genotyping technologies do not confer haplotypes, thus methodologies are needed to estimate population haplotype frequencies and individual haplotypes derived from the observed SNP genotype. The two most popular likelihood-based algorithms are the expectation-maximization (EM) algorithm assuming a multinomial probability model and Hardy-Weinberg proportions, and Clark's parsimony method. A novel Markov chain-Monte Carlo algorithm based approach using Gibbs sampling was recently described as providing a more accurate estimate of individual haplotypes. We have developed a Gibbs sampling methodology for the multinomial Hardy-Weinberg model to enable a more accurate calculation of confidence intervals and probabilities around each estimated haplotype. We describe here a comparison of the EM algorithm and our Gibbs sampling method via computer simulations, and on two actual studies. The first study examines 8 single nucleotide polymorphisms spanning 26 kb of the angiotensin converting enzyme genotyped in a population of 250 subjects. In addition, we also compare the efficiency of each approach in a population of 150 subjects, against a two-polymorphism molecularly defined haplotype within the P2X7R gene.



**Fine mapping of region 1p32 that contains the third major locus for autosomal dominant hypercholesterolemia.**

*M. Abi-Fadel*<sup>1</sup>, *L. Villegier*<sup>1</sup>, *S. Rochaud*<sup>1</sup>, *A. Robin*<sup>1</sup>, *J-P. Rabes*<sup>1,2</sup>, *M. Devillers*<sup>1</sup>, *M. Martinez*<sup>3</sup>, *C. Junien*<sup>1,2</sup>, *C. Boileau*<sup>1,2</sup>, *M. Varret*<sup>1</sup>. 1) INSERM U383, Hopital Necker-Enfants Malades, Paris, France; 2) Laboratoire central de biochimie et de genetique moleculaire, CHU Ambroise Pare, Boulogne, France; 3) INSERM U358, Hopital Saint-Louis, Paris, France.

Autosomal Dominant Hypercholesterolemia (ADH), one of the most frequent hereditary disorders, is characterized by an isolated elevation of LDL particles that leads to premature mortality from cardiovascular complications. It is generally assumed that mutations in LDLR and APOB genes account for ADH, however we have shown that ADH is genetically more heterogeneous. We identified 23 ADH families in which we excluded linkage to LDLR and APOB thus demonstrating the implication of a new locus we named FH3. Genetic linkage was obtained in 6 pedigrees localizing FH3 in a 8 cM interval at 1p32-p34.1. This linkage result has been confirmed by S. Hunt et al. in a Utah pedigree. Taken together, the haplotype data define a 1 cM interval for FH3. By radiation hybrid mapping, 6 candidate genes (FABP3, SCP2, APOER2, PAFAH2, AMPK and EPS15) were located outside this interval demonstrating no identity with FH3. Through an e-mapping approach, we built a BAC contig that spans » 3 Mb and contains 2 small non-overlapping areas. The analysis of the human sequence draft reveals more than 30 genes (cloned and predicted). We are currently identifying the expression pattern of these genes and sequencing them in the FH3 families. Current heterogeneity tests estimate that 19% of 23 non-LDLR/non-APOB ADH families are linked to FH3, indicating the implication of a fourth locus called FH4 that we now have also mapped. Finally, we have also detected the possible involvement of a fifth major gene (FH5).

**A physical and transcript map of a region encoding autosomal recessive syndromal and non-syndromal sensorineural hearing loss on human chromosome 11q25.** *E.A.R. Telford, K. Mahmoud, R.F. Mueller.* Molecular Medicine Unit, University of Leeds, Leeds, Yorkshire, United kingdom.

We have previously described the use of autozygosity mapping in a large consanguineous family to identify a gene locus on human chromosome 11q25 causing histiocytosis with associated features of sensorineural deafness and joint contractures. The form of histiocytosis exhibited by this family does not fit readily into any of the recognised classes of the disease and appears to represent a novel form of familial histiocytosis demonstrating autosomal recessive inheritance. We have mapped the minimal critical region of homozygosity in this family to a 1cM interval encompassed by the markers D11S1309 and D11S968. The existence of a novel non-syndromal autosomal recessive deafness locus (DFNB20) in this region was subsequently demonstrated by linkage analyses of 35 consanguineous families segregating non-syndromal sensorineural hearing loss with microsatellite markers from chromosome 11q25. An approximately 3cM region of homozygosity was observed in four affected individuals from a single family spanning the interval D11S1320-qter.

We are currently continuing this research using a positional candidate gene approach to identify and characterise the responsible gene(s) at this locus. We present a detailed physical and transcript map of the 11q25 region that includes ten known genes. Transcripts expressed in cochlear and/or myeloid tissues will be considered as suitable candidates for mutation screening.

We also report the identification of six new polymorphic markers between D11S1320 and D11S968 which enable us to further define the location of the histiocytosis syndrome and facilitates further fine mapping in this region.

**A major marker for normal tension glaucoma: Association with polymorphisms in the *OPAI* gene.** T. Aung<sup>1,2,3</sup>, L. Ocaka<sup>1</sup>, N.D. Ebenezer<sup>1</sup>, A.G. Morris<sup>1</sup>, P.J. Francis<sup>1</sup>, D.L. Thiselton<sup>1</sup>, C. Alexander<sup>1</sup>, M. Votruba<sup>1,2</sup>, G. Brice<sup>4</sup>, A.H. Child<sup>4</sup>, R.A. Hitchings<sup>2</sup>, O.J. Lehmann<sup>1,2</sup>, S.S. Bhattacharya<sup>1</sup>. 1) Molecular Genetics, Institute of Ophthalmology, University College London, United Kingdom; 2) Moorfields Eye Hospital, London, UK; 3) Singapore National Eye Centre, Singapore; 4) St George's Hospital Medical School, London, UK.

**Background.** Normal tension glaucoma (NTG) is a major form of glaucoma, associated with intraocular pressures that are within the statistically normal range of the population. *OPAI*, the gene responsible for autosomal dominant optic atrophy represents an excellent candidate gene for NTG as the clinical phenotypes are similar, and *OPAI* is expressed in the retina and optic nerve. **Methods.** Eighty-three well-characterized NTG patients were screened for mutations in *OPAI* by heteroduplex analysis and bi-directional sequencing. Exons found to be altered in NTG subjects were examined for variations in 100 population controls. A second cohort of 80 NTG patients and 86 population controls was subsequently screened to determine whether the initial findings could be replicated. **Results.** A total of 163 NTG subjects and 186 controls were included in the study. Thirty-two NTG subjects (19.6%) were found to carry two single nucleotide polymorphisms (SNPs) on intervening sequence (IVS) 8 compared to only 7 (3.7%) control subjects ( $\chi^2 = 22.04$ ,  $p = 8 \times 10^{-6}$ , after correcting for testing four genotypes) indicating that the association of this genotype is very strongly associated with the occurrence of disease. **Conclusions.** Polymorphisms in the *OPAI* gene are associated with NTG. Such changes are present in a fifth of NTG cases, which equates to 4-10% of open angle glaucoma.

**Evaluation of serotonin transporter gene (SLC6A4) polymorphisms in mood disorders with comorbid gastrointestinal abnormalities using temperature modulated heteroduplex chromatography.** *A.R. Belous<sup>1</sup>, L. Brown<sup>2</sup>, R.C. Shelton<sup>2</sup>, H. Metrz<sup>3</sup>, R.D. Blakely<sup>1</sup>.* 1) Dept Pharmacology, 412 PRB, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Dept of Psychiatry, Vanderbilt University, Nashville, TN 37232-8626; 3) Dept of Medicine, Division of Gastroenterology, Vanderbilt University, Nashville, TN 37232-5340.

A major hypothesis regarding the etiology and pathogenesis of mood disorders is that they involve abnormalities of the serotonin (5-HT) system. The serotonin transporter (SERT, SLC6A4) is an important candidate gene for complex disorders linked to altered 5-HT signaling. We are exploiting a candidate gene approach, searching for single nucleotide polymorphisms (SNPs) in SLC6A4 in mood disorders. Our hypothesis is that the same gene and SNPs may be involved in pathogenesis both of primary mood disorders and comorbid disorders involving peripheral physiological responses linked to 5-HT. Major depressive disorder (MDD) often is comorbid with gastrointestinal (GI) disturbances such as Irritable Bowel Syndrome (IBS). The GI tract is rich in 5-HT and SERT proteins that are highly expressed in both epithelial cells and myenteric neurons. Thus, mutations in SERT could support psychiatric disorders with comorbid IBS-like disorders. In our initial study, DNA samples from unrelated individuals with MDD with IBS-like abnormalities and subjects with IBS are being analyzed for SLC6A4 exon and splice junction mutations. PCR amplicons of each SERT exon are analyzed for SNPs using Denaturing High Performance Liquid Chromatography on the WAVE-TM DNA Fragment Analysis System (Transgenomic Inc.), followed by direct DNA sequencing. WAVE analysis of SLC6A4 amplicons has revealed several heterozygous SNPs in exon 1b in patients with either IBS or MDD with IBS-like GI abnormalities. Whether these SNPs or ones in other exons support MDD/IBS will require analysis of additional subjects, careful evaluation of allele frequencies in unaffected populations and functional validation using both SERT-specific antibodies and/or heterologous expression of mutant cDNAs (This work is supported by a NARSAD Young Invest. Award to A.B; DA07390 and MH58293 to R.B; MH01741 to R.S.).

**Polymorphism analysis in the COLIA1 gene of patients with thalassemia major and intermedia.** *E.Y. Fenerci<sup>1</sup>, O. Arisal<sup>2</sup>, N. Sayhan<sup>3</sup>, S. Hacıhanefioglu<sup>1</sup>, S. Erkmén<sup>2</sup>, A. Deviren<sup>4</sup>.* 1) Department of Medical Genetics, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey; 2) Thalassemia Center, Nicosia Dr.Burhan Nalbantoglu State Hospital, Nicosia, Cyprus; 3) Molecular Oncology and Hematopathology Research and Application Center, Istanbul University, Istanbul, Turkey; 4) Genetics and Teratology Research and Application Center, Istanbul University, Istanbul, Turkey.

Beta thalassemia major is an inherited blood disorder, mainly affecting people originating from Mediterranean region. The disease results in a life-threatening anemia and requires regular blood transfusion and iron-chelating therapy throughout life from early childhood. There is a high incidence of osteopenia and osteoporosis in patients with thalassemia major. Genetic factors play an important role in determining bone density, which is a major determinant of osteoporotic fractures. The inheritance of bone mass is under polygenic control. Type I collagen, which is encoded by COLIA1 and COLIA2 genes, is the major protein in the bone. A polymorphism G®T in the regulatory region of COLIA1 gene at a recognition site for transcription factor Sp1 has recently been strongly associated with reduced bone mass and osteoporotic fractures. The G®T polymorphism is screened in 42 beta thalassemia major and 10 beta thalassemia intermedia patients. After COLIA1 gene was amplified by polymerase chain reaction and digested with BalI restriction enzyme, the products were subjected to a 12% polyacrylamide gel electrophoresis. 64.3% of the beta thalassemia major patients were heterozygous for G/T (Ss) polymorphism and 35.7% were homozygous for G/G(SS). 60% of the beta thalassemia intermedia patients were heterozygous (Ss) and 40% were homozygous (SS). The number of heterozygotes in the beta thalassemia major group compared to the control group was significantly more (F=13.165, p=0.001). The number of heterozygotes in thalassemia intermedia group compared to the control group was also significantly more (F=5.158, p=0.029). Patients who are G/T heterozygotes (Ss) at the polymorphic Sp1 site have a lower bone mineral density than G/G homozygotes (SS) (p=0.01).

**Genotyping diallelic insertion/deletion polymorphisms.** *N.R. Ghebraniou<sup>1</sup>, J. Che<sup>1</sup>, D. David<sup>1</sup>, T. Rusch<sup>1</sup>, J. Chudyk<sup>1</sup>, K. Fieweger<sup>1</sup>, B. Dickinson<sup>1</sup>, Y. Fan<sup>1</sup>, C. Zhao<sup>1</sup>, A. Yu<sup>1</sup>, G. Marth<sup>2</sup>, M. Doktycz<sup>3</sup>, J.L. Weber<sup>1</sup>.* 1) Ctr Medical Genetics, Marshfield Medical Res Fndn, Marshfield, WI; 2) National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; 3) Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN.

Over 2000 human diallelic insertion/deletion polymorphisms have been confirmed and characterized to date at Marshfield using overlapping genomic and cDNA sequences. Overlapping BAC sequences have been the richest source of markers. A searchable database of the indels is available from the Marshfield website ([research.marshfieldclinic.org/genetics](http://research.marshfieldclinic.org/genetics)). For analysis of the indels, we have developed an efficient, homogenous, closed-tube system involving allele-specific PCR and molecular beacons. Molecular beacons are synthetic oligos with a stem/loop structure containing both fluorescent dye and quencher molecules. When the stem is closed, fluorescence is quenched. When the stem is opened, the dye and quencher molecules are physically separated, and fluorescence is dramatically increased. The beacons used in our indel assay have dye-specific universal tails of about 18 bases at the 3' ends that allow them to be incorporated into PCR products. Using two different molecular beacons labeled with Fam and Joe dyes, we can simultaneously detect both alleles in the same reaction. Currently, we carry out the assay in 384 well microtiter plates using reaction volumes as low as 2 ml. In the near future, microtiter plates will be replaced by microtape. Microtape is a continuous, wide plastic strip embossed with periodic 384 well patterns. The price of microtape is about 12% the cost of plates. Microtape also contains index holes along each side, which permit sprocket-driven translation of the tape through pipetting and scanning instruments. Our engineering team has developed several versions of fluorescence scanners for both plates and tape. The latest version will scan a 384 well pattern on the tape in about 10 min. Images from the scanners are processed using software also developed at Marshfield. Through use of the tape and through reduction in reaction volumes, we expect to steadily drop diallelic polymorphism genotyping costs.

**Identification of coding single nucleotide polymorphisms (cSNPs) in genes expressed in tumoral cells using the HCGP-ORESTES database.** *M.C.R. Costa<sup>1</sup>, W.A. Silva Jr<sup>1,2</sup>, A.J. Holanda<sup>1,4</sup>, I. Tojal<sup>1</sup>, E. Dias-Neto<sup>3</sup>, P.E.M. Guimaraes<sup>3</sup>, E.P.B. Ojopi<sup>3</sup>, S.J. Souza<sup>3</sup>, A.J.G. Simpson<sup>3</sup>, M.A. Zago<sup>1,4</sup>.* 1) Molecular Biology and Bioinformatics Laboratory - Center for Cell-Based Therapy, Blood Center of Ribeirao Preto; 2) Federal University of Para - Santarem Branch; 3) Ludwig Institute for Cancer Research (LICR) - Sao Paulo Branch; 4) Medical School of Ribeirao Preto - University of Sao Paulo, Brazil.

Single Nucleotide Polymorphisms (SNPs), the most common type of variation in the human genome, correspond to positions where two alternative bases occur with appreciable frequency in the human population (>1%). Although most of these variations have no clinical or biological significance, an increasing number of single base mutations is being identified as responsible for the molecular basis of genetic disease and cancer or as risk factors for acquired diseases. In this study, we developed an approach to identify cSNPs in genes expressed in tumors using the HCGP-ORESTES database (Human Cancer Genome Project - LICR-FAPESP - Brazil). To this end, it was established a pipeline using the programs Phred, cross\_match and PolyBayes, an algorithm that detects SNPs eliminating paralogs and variations resulting from sequencing errors. Using the PolyBayes output, a user-friendly report with the detailed information about each candidate cSNP was generated, including the flanking region of the variation. In order to include cSNPs already reported, we also performed BLAST searches in the dbSNP. Using this approach, a set of 167,195 ORESTES corresponding to 7,950 human genes was analysed. Only the cSNPs that were detected in two or more different libraries and those present in the dbSNP were computed in the final statistics. A total of 554 candidate cSNPs were identified, being 345 non-synonymous (210 transitions, 135 transversions), and 209 synonymous (163 transitions, 46 transversions). From these, 48 showed to be already deposited in dbSNP. Thus, an approach to identify SNP was developed and performed in silico, a local database was created and experimental validation is in course in our laboratories.

**Association of *NOS1* gene polymorphisms with atopy and eosinophil count in 107 French EGEA families.** *F.M. Demenais<sup>1</sup>, M. Boussaha<sup>1</sup>, M.H. Dizier<sup>2</sup>*. 1) INSERM EMI 00-06, Evry; 2) INSERM U535, Kremlin-Bicêtre, France.

The *NOS1* gene is mapped to 12q24, a region found linked to asthma-related phenotypes by several genome screens. Our screen, conducted in 107 French EGEA families with at least two asthmatic sibs, showed suggestive evidence for linkage of eosinophil count in the vicinity of *NOS1* ( $p = 0.0007$ ). Our goal was to investigate the role of *NOS1* in asthma-related phenotypes in our family sample.

Three multi-allelic polymorphisms within exon 29, intron 2 and promoter region of *NOS1* were genotyped in 212 parents and 279 offspring of the 107 EGEA families. Linkage and association of these variants with asthma phenotypes were investigated using 2 approaches: (1) Transmission Disequilibrium Test (TDT) for binary traits (asthma, MultiRAST Phadiatop, Skin Prick Test (SPT) positivity to at least one of 11 allergens, SPT to Timothy Grass Pollen and SPT to *Dermatophagoides pteronyssinus*, Der p) and (2) combined segregation-linkage analysis based on regressive models for quantitative traits (Immunoglobulin E levels, eosinophil count, slope of dose-response curve to methacholine). TDT-based analyses indicate an excess of transmission of 187 bp allele in intron 2 from parents to offspring affected with either one of three atopy-related phenotypes (SPT, MultiRAST, SPT to Der p), the  $p$ -value being 0.01 for each trait ( $pc = 0.05$  after correction for multiple testing). Preliminary analyses of quantitative traits, using the Man-Whitney test, lead to a significant association of allele 18 of a CA repeat polymorphism in exon 29 with eosinophils, especially in non-asthmatics ( $p = 0.001$ ;  $pc = 0.004$ ). Combined segregation-linkage analysis incorporating linkage disequilibrium confirms this result by showing evidence for an effect of allele 18 of exon 29 on eosinophil count, when considering only non-asthmatic subjects ( $p = 0.01$ ). This variant accounts for 4% of eosinophils variation. This result agrees with the decreased risk of asthma associated with the same allele reported by a recent case-control study.

Supported by INSERM-MSD and IDS.



**Association studies of CTLA-4, CD28 and ICOS gene polymorphisms with type 1 diabetes in the Japanese population.** *K. Ihara<sup>1</sup>, S. Ahmed<sup>1</sup>, F. Nakao<sup>1</sup>, N. Kinukawa<sup>2</sup>, R. Kuromaru<sup>1</sup>, N. Matsuura<sup>3</sup>, I. Iwata<sup>4</sup>, S. Nagafuchi<sup>4</sup>, H. Kohno<sup>5</sup>, K. Miyako<sup>5</sup>, T. Hara<sup>1</sup>.* 1) Dept Ped, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan; 2) Dept Med Inform, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan; 3) Dept Ped, Kitasato Univ Sch Med, Sagamihara, Japan; 4) Dep Med Biosys Sci, Grad Sch Med Sci, Kyushu Univ, Fukuoka, Japan; 5) Dep Endocrinol Metab, Fukuoka Child Hosp, Fukuoka, Japan.

Co-stimulatory molecules of CD28, Cytotoxic T-lymphocyte associated antigen-4 (CTLA-4), and newly identified Inducible costimulator (ICOS), are expressed on cell surfaces providing regulatory signals for T-cell activation. These genes are candidate susceptibility genes for type 1 diabetes because they are colocalized on chromosome 2q33 including IDDM 12 locus. After determination of the genomic structure and screening for polymorphisms of ICOS gene, we performed association studies between newly identified polymorphisms of ICOS gene or the known ones of CD28 and CTLA-4 genes, and type 1 diabetes. The 49A/G dimorphism in the exon 1 and the (AT)<sub>n</sub> in the 3' untranslated region of CTLA-4 gene were significantly associated with type 1 diabetes. The evaluation of CTLA-4 49A - 3' (AT)<sub>n</sub> 86 bp haplotype frequency in the patients and controls confirmed the results from the analysis of each polymorphic site. Dimorphism in the intron 3 of CD28 gene was associated with type 1 diabetes only in the early onset group. In contrast, there was no association of the microsatellite polymorphisms in the ICOS gene or dimorphisms in the promotor region of CTLA-4. In conclusion, CTLA-4 gene appears to confer risks for the development of type 1 diabetes among three genes encoding costimulatory molecules.

**Frequency of the vCJD-associated M/M 129 PRNP genotype in the Quebec population.** *D. Jung*<sup>1, 2</sup>, *M. Drouin*<sup>1</sup>, *R. Lemieux*<sup>1</sup>. 1) Dept Research & Development, Hema-Quebec, Sainte-Foy, Quebec, Canada; 2) Laval University, Quebec, Quebec, Canada.

The occurrence of new variant-Creutzfeldt Jakob disease (vCJD) in the UK, has raised concerns that asymptomatic blood donor could contaminate the blood supply. To reduce the theoretical risk of transmission of the vCJD prion agent by blood transfusion, the north american blood agencies have introduced a blood donor selection criteria based on length of UK or France residency. The maximal residency period was selected after consideration of regional differences in the travel frequency to UK or France among blood donors (1 or 6 months) and of maximal tolerable impact on blood collection (<5%). The extensive testing done so far on tissues of vCJD patients in the UK has revealed that a genetic factor appears to predispose individuals to develop vCJD. The PRNP gene encoding the normal prion protein has several polymorphisms including the methionine(M) or valine (V) at codon 129. Published data indicate that all vCJD patients tested are M/M homozygous at codon 129 of their PRNP gene while the M/M genotype frequency is only 38% in the European population (V/V 11%, M/V 51%). As a first step in the evaluation of the possible usefulness of PRNP genotyping in donor exclusion, we have set-up a simple laboratory assay for PRNP genotyping at codon 129. The entire coding sequence of the PRNP gene was amplified by PCR and cut with the NspI endonuclease which is only able to digest the amplicon generated from the M129 PRNP gene. Separation of the NspI digested PCR products by gel electrophoresis allows an easy identification of the codon 129 genotype. Sequencing of the amplicons from homozygous and heterozygote individuals has confirmed the M/M, V/V and M/V genotypes. Results obtained with 341 volunteers indicate that the frequency of the M/M genotype (39.5%) appears similar to the one in Europe (38%) V/V and M/V genotypes frequency are respectively 12.5 and 48%. The routine PRNP genotyping of blood donors could permit to strenghten the exclusion criteria currently used.

**Difference in linkage disequilibrium in 300 kb region around smoothelin gene (chromosome 22) among 3 ethnically different populations.** *H. Morisaki*<sup>1</sup>, *Y. Kitamura*<sup>2</sup>, *N. Kamatani*<sup>2</sup>, *T. Morisaki*<sup>1,3</sup>. 1) Dept Bioscience, Natl Cardiovasc Ctr Res Inst, Suita, Osaka, JAPAN; 2) Inst Rheumatol, Tokyo Women's Med Univ, Tokyo, JAPAN; 3) Dept Mol Pathophysiol, Osaka Univ Grad Sch Pharm Sci, Suita, Osaka, JAPAN.

Single nucleotide polymorphisms (SNPs) and short tandem repeat polymorphisms (STRPs) are thought to be powerful tools for the genome-wide linkage disequilibrium (LD) analysis to map traits. However, the success of the analysis depends on the strength and extent of LD between the disease allele and the markers. Although the extent of LD in general population is of interest, little attention has been paid to the differences among ethnically different populations. In this study, 76 SNPs and 2 STRPs around human smoothelin gene scattered over 300 kb region were analyzed in 32 subjects each from Japanese, European American and African American populations. SNPs shared by these three populations were generally present at higher frequencies than unshared SNPs, and LDs between shared SNPs revealed more prominent decay of LD as a function of distance compared to those between SNPs at least one of which is unshared. Japanese exhibited the shortest span of LD in this region, while European Americans showed the longest strength and extent of LD between SNPs differed between the populations, suggesting that SNPs and STRPs for LD analysis should be selected for each population. Analysis of STRPs in the context of the haplotypes constructed by SNPs suggested that STRP polymorphisms are generally younger than SNPs. Whether STRPs are useful for LD analysis for finding disease-related mutations probably depends on when the disease-related mutations occurred.

**Strategies for polymorphism discovery in a disease specific population.** A. Loukola<sup>1</sup>, M. Chadha<sup>1</sup>, B. Nguyen<sup>1</sup>, S.G. Penn<sup>1</sup>, D.R. Rank<sup>1</sup>, D.K. Hanzel<sup>1</sup>, G. Casey<sup>2</sup>, J.S. Witte<sup>3</sup>. 1) Aeomica Inc., Sunnyvale, CA; 2) Department of Cancer Biology, Cleveland Clinic Foundation, Cleveland, OH; 3) Case Western Reserve University, Cleveland, OH.

Single nucleotide polymorphisms (SNPs), are expected to be helpful in identifying human disease genes. Finding SNPs, however, is just the beginning. They have to be confirmed as true variations and their frequencies in different populations need to be determined. Current estimates are that SNPs occur every 300-800 bases along the chromosomes. This density should provide detailed coverage of the genome and allow researchers to locate regions of interest more precisely, by narrowing in on phenotypic loci. In addition, SNPs, or perhaps combinations of them, may also affect gene function and activity. There are a number of strategies for identifying disease important SNPs, and we have examined these strategies. We studied two Cytochrome P450 genes, CYP17 and CYP3A4. We sequenced PCR products covering these two genes in two populations, using a MegaBACE 1000 high-throughput DNA sequencer. One population was 110 individuals from a prostate cancer sibship study (CaP), and the second population was 24 individuals from the Coriell Diversity Set (CDS). In addition we looked for previously discovered SNPs in the public databases. Following SNP discovery, we went on to genotype all the SNPs in both populations, using a single base extension assay (SNUPe). These experiments were carried out to confirm the true frequencies of the SNPs in the different populations. To date we have found a total of 14 novel SNPs in CYP17, and 23 novel SNPs in CYP3A4. In this paper we give a full analysis of the intersection of the SNP frequency found by the different strategies and discuss the merits of using public SNP databases. We conclude that while a large number of SNPs can be discovered using a generic population (CDS), a disease specific population provides unique SNPs. Whether disease specific SNPs turn out to be crucial in disease mapping or have an effect on the phenotype remains to be seen. Our ongoing study aims at finding association between SNPs and an aggressive form of prostate cancer.

**Genetic variation in the matrix metalloproteinase 9 gene, MMP9, in Type 2 diabetes patients with renal disease.**

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Matrix metalloproteinase 9 (MMP9) in 20q12-q13.1 is secreted by glomerular mesangial cells and increased in renal diseases such as membranous nephropathy and renal fibrosis suggesting an association with renal disease. Linkage and association studies in Caucasians with Type 2 diabetes (T2DM) associated nephropathy suggest that at least one T2DM gene is located in 20q12-13.1 (Bowden et al., *Diabetes*, 46:882-886, 1997). We scanned 2.2kb of the promoter and all 13 exons (3.3 kb) of MMP9 for sequence variation in Caucasian and African American (AA) T2DM patients with renal disease. SSCP and denaturing high performance liquid chromatography were used to scan for polymorphisms. Differences were confirmed by direct DNA sequencing. A total of 9 variable sites: 4 in the promoter, 4 in the coding region (A20V exon 1; R279Q exon 6; P574R exon 10; G607G exon 11) and one in the 3' untranslated region were found in Caucasians. 14 variable sites: 8 promoter, 5 coding region (A20V, G15G exon 1; R279Q exon 6; P574R exon 10; G607G exon 11) and one in 3' were found in AAs. Three coding region polymorphisms were observed in both populations. The most common variants in Caucasians were the promoter C-1919T (0.68, 0.32), R279Q (0.30, 0.70), G607G (0.36, 0.64) in coding region and C+6T (0.40, 0.60) in 3' and those in AAs were C-2012T (0.68, 0.32), C-1919T (0.72, 0.28) in promoter, R279Q (0.33, 0.67), G607G (0.59, 0.41) in coding region and C+6T (0.79, 0.21) in 3'. The frequencies of these alleles were compared between patient (T2DM nephropathy) and control populations using the Chi-square test. No significant differences were observed between cases and controls, but 4 differences were observed between Caucasians and AAs: C-2012T (seen one AA patient), P574R ( $p < 0.01$ ), G607G ( $P < 0.01$ ) and C+6T ( $P < 0.01$ ). We have compared our results with those of a similar study in Caucasians (Zhang et al., *Hum Genet* 105, 418-423, 1999). Results were similar in the two studies except we did not see the T-1702A seen by Zhang et al. and they did not find P574R. They also did not observe the C-2012T variant.

**Brain Derived Neurotrophic Factor (BDNF) Is Associated With Childhood Onset Depression.** *N. King*<sup>1</sup>, *C. Barr*<sup>2</sup>, *S. Shaikh*<sup>1</sup>, *B. Devlin*<sup>3</sup>, *K. Wigg*<sup>2</sup>, *M. Kovacs*<sup>3</sup>, *J.L. Kennedy*<sup>1</sup>. 1) Neurogenetics Section, Ctr Addiction & Mental Health, Toronto, ON., Canada; 2) University Health Network, Ontario, Canada; 3) University of Pittsburgh, Pittsburgh, U.S.A.

Childhood onset depression (COD) is associated with significant morbidity, and has been repeatedly shown to have high familial loading. Family studies have found that between 40% and 70% of first-degree relatives of depressed children have a history of major depressive disorder and bipolar illness, compared to a significantly lower rate in relatives of healthy juvenile controls. An interesting candidate locus for COD is brain-derived neurotrophic factor (BDNF), a neurotrophin abundant in the neocortex, amygdala and hippocampus, responsible for neuronal proliferation, differentiation and survival. In animal studies, injection of BDNF provides evidence of an antidepressant effect. We hypothesize that variants at the BDNF locus causing decreased gene expression are associated with a vulnerability to depression. The human BDNF gene maps to 11p13-15. In a sample of 77 COD patients, diagnosed using the DSM-IV, and healthy volunteers matched for ethnicity and sex, we genotyped a dinucleotide repeat polymorphism located 1040 bp upstream of the transcription site, and a G®A (valine®methionine) variation in position 758 of the coding sequence. The dinucleotide repeat showed a significant association with COD with p-value = 0.000035,  $c^2 = 25.759$ , df = 4. Allele 4 (168 bp) conferred a relative risk of 8.0 for child depression (95% C.I. 2.388-33.114), with odds ratio of 9.29 (95% C.I. 2.584-39.700). Results for the G®A polymorphism are  $c^2 = 0.749$ , df = 1, and p = 0.387. Thus far, our results suggest that the BDNF gene may play a role in the etiology of COD. Additional studies are warranted in larger samples, and using alternative strategies such as genomic controls and TDT.

**Determination of human  $\beta_2$ -adrenergic receptor haplotypes by denaturation selective amplification and subtractive genotyping.** *J. Wang<sup>1</sup>, L.M. Humma<sup>2</sup>, E.B. Mougey<sup>1</sup>, C.J. David<sup>1</sup>, J.A. Johnson<sup>2</sup>, J.J. Lima<sup>1</sup>, J.E.*

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The  $\beta_2$  adrenergic receptor ( $\beta_2$ -AR) gene contains single nucleotide polymorphisms (SNPs) at nucleotides -47 (5' Leading Cistron-Cys/Arg-19), 46(Gly/Arg16) and 79(Gln/Glu27) that have been implicated in numerous diseases and/or their modification. Recently, Ulbrecht et al. reported that the Gly16/Gln27/Thr164 haplotype, *but not genotype*, protected women against bronchial hyper-responsiveness. Conventional methods for obtaining haplotype information (i.e. cloning and sequencing or using polymorphism specific primers for amplification) are laborious and time consuming. Thus we sought to develop an alternative method for obtaining haplotype information at positions -47, 46, and 79 of the  $\beta_2$ -AR gene. Inspection of the  $\beta_2$ -AR gene sequence revealed that a region surrounding codon -19 was extremely GC rich and thus melting temperature calculations suggested that this region would determine the denaturation temperature required for successful amplification of the entire gene. In addition the C/G base pair of the Arg-19 allele was found to increase the local melting temperature over the T/A base pair of the Cys-19 allele by 3.6 degrees and establish a new local maximum denaturation temperature. We confirmed this difference in denaturation temperature and found that Cys-19 alleles were selectively amplified at a denaturation temperature of 94°C while the Arg-19 allele required a minimum denaturation temperature of 95°C. Using a combination of this Denaturation Selective Amplification (DSA) and conventional RFLP analysis we were able to successfully haplotype a total of 107 patients and predict that this method will be useful for > 99% of the population at these three SPNs. In addition our observation of DSA suggests caution when analyzing genotyping data and provides a possible explanation for allele dropout during amplification.

**A high speed and large-scale single nucleotide polymorphism (SNP) typing system.** *a. sekine*<sup>1</sup>, *h. suzuki*<sup>2</sup>, *k. hirotani*<sup>2</sup>, *k. jinno*<sup>3</sup>, *t. kitamoto*<sup>1</sup>, *t. sakamoto*<sup>1</sup>, *t. utagawa*<sup>3</sup>, *t. matsumura*<sup>3</sup>, *y. ohnishi*<sup>4</sup>, *t. tanaka*<sup>4</sup>, *y. nakamura*<sup>1,2</sup>. 1) Lab. for Genotyping SNP Res. Cent. RIKEN, Tokyo, JAPAN; 2) Lab. of Mol. Med. Human Genome Cent. Ins. of Med. Sci., Tokyo Univ. ,Tokyo, JAPAN; 3) Jap. Biol. Informatics Consortium, Tokyo, JAPAN; 4) Lab. for Cardiovascular diseases RIKEN, Tokyo, JAPAN.

Single nucleotide polymorphisms (SNPs) are single-base variations that occur at a frequency of approximately one per every 300-1000 bp throughout genome. SNPs are useful genetic tools to uncover the loci containing genes associated with or susceptible to diseases. Since each of common diseases is considered to have associations with 10-20 genetic loci, it is inevitable to screen SNPs throughout the genome in a large number of individuals for identification of susceptible genes as many as possible. To develop a high-speed and large-scale SNP typing system, we recently established a novel technology by a combination of multiplex PCR and the Invader SNP analysis method. The process for genotyping is as follows; (1) multiplex PCR amplification of 96 genomic fragments containing SNPs in one tube, (2) dividing the PCR products for 384 individuals into 96 384-well cards, (3) spotting a reaction mixture corresponding to each SNP to each of the cards using an ink-jet spotter, (4) complete sealing of the card by ultrasonic welding, (5) incubation of the sealed cards at 65°C, and (5) judging genotypes by detection of two fluorescent colors. The multiplex PCR amplification and the sealed card that we developed significantly reduced the amount of genomic DNA necessary for genotyping to 0.1-0.2 ng/SNP as well as the cost for genotyping. The system we set up on the basis of these technologies can achieve about 100 millions SNPs per year, making the genome-wide SNPs screening realistic.



Program Nr: 2115 from the 2001 ASHG Annual Meeting

**Multiplex genotyping of polymorphisms using Pyrosequencing™.** *A. Schiller, J. Dunker, S. Eriksson, A. Alderborn.*  
Pyrosequencing AB, Uppsala, Sweden.

Pyrosequencing™, or real-time sequencing, is a fast and accurate method for SNP analysis. Pyrosequencing AB (Sweden) manufactures the PSQ96™ System, in which 96 different SNPs are analyzed in parallel in approximately 10 min. A dedicated SNP scoring software automatically delivers genotype and a quality assessment for each sample.

In this study, the applicability of pyrosequencing for multiplex genotyping was investigated.

SNPs from the Renin-Angiotensinogen-Aldosterone System (RAAS) were analyzed using pooled simplex PCR products followed by multiplex genotyping. To further enhance cost efficiency in SNP analysis, multiplex PCR followed by multiplex genotyping by pyrosequencing was tested using the Factor V Leiden, Prothrombin G20210A and the PAI1 4G/5G polymorphisms. A third approach was to design several sequencing primers for one PCR fragment containing a number of different polymorphisms, illustrated by allele identification in CYP2D6. All three strategies (pooled simplex PCR/multiplex pyrosequencing, one single PCR fragment/several sequencing primers and multiplex PCR/multiplex pyrosequencing) resulted in highly reproducible and accurate scores, which were in complete accordance with those obtained when genotyping each polymorphism independently. Design of sequencing primers in combination with a reaction specific dispensation order enabled separate typing of the polymorphic positions whilst keeping the genotyping quality identical to that of simplex genotyping. In addition, this approach allowed unequal amplification efficiency to occur without negatively affecting the genotyping results. The sequences surrounding the SNPs and deletion polymorphisms confirmed the correct positioning of the primers on their respective gene sequences. Thus, pyrosequencing enables reliable and robust analysis of several polymorphisms in a single pyrosequencing reaction.

**Interspecies comparisons of the ATM gene.** *T.L. Wayne, Y.R. Thorstenson, P.J. Oefner.* Stanford Genome Technology Center, Stanford University, Palo Alto, CA.

ATM is the gene mutated in the recessive autosomal disease ataxia-telangiectasia. It is also implicated in several types of cancer, making it a putative tumor suppressor gene. To determine the level of selection operating on ATM, we have analyzed the sequence diversity of six species: human (93 individuals), chimpanzee (6 individuals), gorilla (3 individuals), orangutan (3 individuals), New World monkey (1 individual), and Old World monkey (1 individual). PCR was performed using primers designed to the human sequence and all samples were sequenced using a dye terminator reaction. The McDonald-Kreitman test was used to determine the significance of the sequence divergence between species. Specifically, the number of replacement and synonymous mutations found within species (polymorphism) was compared to that found between species (divergence). The neutral evolution model predicts that the ratio of replacement to synonymous differences is the same in divergent sites as it is in polymorphic sites. If this assumption fails, then it is likely that the gene in question is under selective pressure. Whereas most comparisons across species revealed no difference, four revealed significant deviation from the neutral expectation: human compared to gorilla and orangutan, and orangutan compared to gorilla and monkey. Within most species, the synonymous polymorphisms were more numerous than the replacement polymorphisms. Likewise, the Hudson, Kreitman, Aguade (HKA) test was applied to compare the levels of polymorphism and divergence at different loci. In this case, the neutral evolution model predicts that the relationship of polymorphism to divergence is the same for different genes, whereas selection can lead to an uncoupling of polymorphism and divergence. We compared the first 71% of the ATM gene with the last 29%, which contains the conserved kinase, Rad3-like, and Tel1p-like domains. The comparison revealed that the carboxy terminus of the human ATM gene was significantly constrained relative to orangutan, Old World monkey and mouse, but not to chimpanzee or gorilla. Our findings from both tests indicate that ATM is under significant selective pressure.

**Age-related maculopathy (ARM): An expanded genome-wide scan with evidence of susceptibility loci within the 1q31 and 17q25 regions.** *M.B. Gorin<sup>1,2</sup>, D.E. Weeks<sup>1</sup>, Y.P. Conley<sup>1,3</sup>, H.-J. Tsai<sup>1</sup>, T.S. Mah<sup>2</sup>, P.J. Rosenfeld<sup>4</sup>, T.O. Paul<sup>5</sup>, A.W. Eller<sup>2</sup>, L.S. Morse<sup>6</sup>, J.P. Dailey<sup>7</sup>, R.E. Ferrell<sup>1</sup>.* 1) Dept. of Human Genetics, Univ. of Pittsburgh GSPH, Pittsburgh, PA; 2) Dept. of Ophthalmology, Univ. of Pittsburgh SOM, Pittsburgh, PA; 3) Dept. of Health Promotion & Development, Univ. of Pittsburgh SON, Pittsburgh, PA; 4) Dept. of Ophthalmology, Univ. of Miami, Bascom Palmer Eye Inst., Miami, FL; 5) Smith-Kettlewell Eye Research Institute, San Francisco, CA; 6) Dept. of Ophthalmology, University of California Davis, Davis, CA; 7) Dept. of Ophthalmology, Case Western Reserve, Cleveland, OH.

We seek to identify genetic loci that contribute to ARM susceptibility by employing a family -based cohort study for linkage analysis. ARM families consisting of at least two affected siblings were ascertained using eye care records and fundus photographs. Additional family members were used to increase the power to detect linkage. Microsatellite genotyping was conducted by the NHLBI Mammalian Genotyping Service and the NIH Center for Inherited Disease Research. Linkage analyses were conducted with parametric (autosomal dominant) (heterogeneity lod score HLOD) and nonparametric methods ( $S_{all}$  statistic) using three diagnostic models. False positive rates were determined from simulations using actual pedigrees and genotyping data. With our combined set of 391 ARM families, four regions, 1q31, 9p13, 10q26, and 17q25, showed multipoint HLODs or  $S_{all}$ 's 2.0 (under at least one model). Under our most stringent model, the 1q31 HLOD was 2.46 between D1S1660 and D1S1647. Under Model C, the 17q25 HLOD at D17S928 was 3.16. The 1q31 region independently confirms the ARMD1 locus reported by Klein et al. Simulations indicate that the 1q31 and 17q25 loci are unlikely to be false positives. An additional 126 individuals (from prior or new families) were genotyped with the same markers around our four peak signals. Fifteen new markers for regions 1q31 and 17q25 have been genotyped on our entire set of 920 individuals to achieve an average of 2-3 cM spacing. The additional genotyping data should aid in our refinement of the candidate regions.

**Autosomal dominant Stargardt disease: clinical features and linkage analysis in a large Greek pedigree. G.**

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Stargardt disease (STGD) is the most common hereditary macular dystrophy and is characterized by decreased central vision during the first decades of life, atrophy of the macula and underlying retinal-pigment epithelium, and presence of yellow spots distributed in the posterior pole of the retina (fundus flavimaculatus). In most instances STGD is inherited as an autosomal recessive trait, and linkage analysis assigned the STGD1 locus to chromosome 1p, due to mutations in the ABCR gene. Several families have been described in which features of STGD are transmitted in an autosomal dominant manner, and dominant loci have been mapped to chromosomes 6q (STGD3, ELOVL4 gene) and 4p (STGD4). We describe a large five-generation pedigree with STGD from Epirus, Greece, comprising 234 individuals (23 affected). Affected individuals of both sexes were found in each generation, consistent with autosomal dominant transmission. Clinical examination revealed visual acuity ranging from 1/50 to 8/10, with age at onset from 7-18 years. Fundoscopic examination showed loss of macular reflex, beaten bronze appearance of the foveal region, and white-yellow spots (flecks) of varying degree. A red-green dyschromatopsia was characteristic. The fluorescein angiography demonstrated areas of dark choroid, window defects, and typical flavimaculatus flecks. The disease was symmetric in the two eyes and progressive with age. Linkage analysis with short tandem repeat (STR) markers excluded linkage to the RDS, STGD3 and STGD4 loci, and a genomewide search is being performed. This family therefore probably represents a new dominant STGD locus.

**Identification of novel exons and analysis of autism-associated sequence variations within the chromosome**

**15q11-q13 region.** *S. Ichikawa*<sup>1</sup>, *Y. Liu*<sup>1</sup>, *J.H. Miles*<sup>2</sup>, *R.E. Hillman*<sup>1,2</sup>, *C.H. Wang*<sup>1,2,3</sup>. 1) Department of Biochemistry; 2) Department of Child Health; 3) Departments of Psychiatry and Neurology, University of Missouri-Columbia, Columbia, MO.

Autism is a neurodevelopmental disorder with core symptoms including impaired social and language development and repetitive, stereotypic behaviors. Previous observations of autism-specific chromosomal abnormalities and suggestive linkage studies indicate that the chromosome 15q11-q13 region may contain an autism susceptibility gene. Recently, we observed an abnormally high rate of meiotic recombination occurring in a small interval between markers D15S986 and D15S1234 in individuals with autism. We also reported the identification of a novel transcript in this region, which is in linkage disequilibrium with autism. To further identify autism candidate genes in this region, we performed exon-trapping experiments using a genomic PAC clone that contains the marker D15S1234. We identified seven independent exons (size 86-120 bp) from this genomic fragment. All seven exons consist of open reading frames and are flanked by conserved splice site sequences. All of the exons have been mapped to the genomic region by comparing to the PAC clone sequence. BLASTN searches detected no homologous sequences in the databases, suggesting that these exons are parts of novel transcripts. Initial mutation analysis using single-strand conformation polymorphism (SSCP) assay in a large group of autism and control samples (N>100) revealed several sequence variations. A total of eight polymorphisms were identified within three exons. One small exon (90 bp) and its flanking 30-bp sequences contain seven polymorphisms, indicating that this region is genetically highly variable. Analysis of allele frequencies detected no significant difference between autism and non-autism controls. Further studies are being conducted using these polymorphic alleles to perform haplotype analysis. We are also obtaining the parental genotypes for a transmission disequilibrium test (TDT). Northern blot analysis will be used to determine the size of these transcripts and their tissue-specific expressions.

**Comparisons of the human and murine pathways in maternally-transmitted deafness.** *Y. Bykhovskaya*<sup>1</sup>, *K.R. Johnson*<sup>2</sup>, *H. Yang*<sup>1</sup>, *K. Taylor*<sup>1</sup>, *R.Y.M. Tun*<sup>1</sup>, *N. Fischel-Ghodsian*<sup>1</sup>. 1) Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA; 2) The Jackson Laboratory, Bar Harbor, ME.

The interaction between the mitochondrial and nuclear genomes has been implicated in the development of maternally-transmitted non-syndromic hearing loss in men and mice. A modifier locus on chromosome 8p influences the severity of hearing loss in families with the A1555G mutation in the mitochondrial 12S rRNA gene, and a single adenine insertion in the mitochondrial tRNA-Arg gene influences the severity of hearing loss in mice homozygous for the abnormal allele at the Ahl (age-related hearing loss) locus. We tried to establish whether the mouse region syntenic to the human chr.8 modifier locus influences hearing loss in the A/J-Cast/Ei backcross model, and whether the human region syntenic to the mouse chr.10 Ahl locus influences hearing loss in the human patients with the A1555G mutation. The human modifier gene corresponds to regions on mouse chromosomes 8 and 14. Genetic analysis of the (A/JxCAST)xA/J backcross mice for mouse chromosome markers D8Mit58, D8Mit191, and D14Mit203 was performed. No evidence of a nuclear effect on hearing was found in either of the two regions. The syntenic region of the mouse Ahl locus on human chr.10 was evaluated in 28 Arab-Israeli, Spanish/Italian and Finnish families with maternally inherited non-syndromic deafness. Multipoint nonparametric linkage analysis in all families combined and in 19 chromosome 8 linked families showed no indication of linkage, while the Lod score in the 9 chromosome 8 unlinked families resulted in positive but non-significant value of 1.0. We also tested three polymorphic markers within the human deafness gene in this region, cadherin 23, in all families combined, and in chromosome 8 linked and unlinked subsets by TDT. Single marker analysis as well as haplotype analysis showed no evidence for linkage disequilibrium. These data therefore do not provide any evidence that the homologues of the mouse and human nuclear regions are involved in the maternally transmitted hearing impairment of the other species. This work was supported by NIH/NIDCD grant no. RO1DC01402 and RO1DC04092 (NFG) and contract DC62108 (KRJ).

**Prevalence of mtDNA mutations in childhood deafness in the Greek population.** *M. Grigoriadou<sup>1</sup>, A. Pampanos<sup>1</sup>, T. Iliades<sup>2</sup>, N. Voyatzis<sup>2</sup>, N. Eleftheriades<sup>2</sup>, V. Iliadou<sup>2</sup>, J. Economides<sup>3</sup>, P. Leotsakos<sup>3</sup>, P. Neou<sup>4</sup>, M. Tsakanikos<sup>4</sup>, L. Katsichti<sup>4</sup>, E. Papadopoulou<sup>5</sup>, E. Kleomitis<sup>1</sup>, J. Gyftodimou<sup>1</sup>, A. Skevas<sup>6</sup>, M.B. Petersen<sup>1</sup>.* 1) Genetics Dept, Inst Child Hlth, Athens, Greece; 2) Aristotle Univ Thessaloniki, Greece; 3) "Aghia Sophia" Children's Hosp, Athens, Greece; 4) "P & A Kyriakou" Children's Hosp, Athens, Greece; 5) Univ Gen Hosp Crete, Heraklion, Greece; 6) Univ Ioannina, Greece.

Genetic factors constitute the major etiology of childhood hearing impairment. The majority of cases are non-syndromic and sensorineural and can be caused by defects in genes located both in the nuclear and mitochondrial genomes. Mitochondrial DNA (mtDNA) mutations such as A1555G, A7445G and 7472insC have been reported in several unrelated families, but a more precise estimate of the prevalence of these mutations as causes of non-syndromic, sensorineural childhood hearing impairment has not been well established. In a collaboration with the major referral centers for childhood deafness in Greece, we therefore screened 261 unrelated patients with congenital/childhood onset sensorineural hearing loss for the known mtDNA mutations A1555G in the 12S rRNA gene and A7445G and 7472insC in the tRNA Ser(UCN) gene. DNA from whole blood was analyzed for the mtDNA mutations using PCR-RFLP analysis. The homoplasmic A1555G mutation was detected in two cases, one sporadic case and one family with affected members in both paternal and maternal lineages. We conclude that routine screening for mtDNA mutations in congenital/childhood onset deafness is warranted, even when maternal inheritance has not been clearly established, the identification of an mtDNA mutation being vital for genetic counseling.

**PREVALENCE OF MITOCHONDRIAL MUTATIONS IN FAMILIAL SENSORINEURAL HEARING**

**IMPAIRMENT : IMPORTANCE OF A1555G AND T7511C.** *D. Feldmann<sup>1</sup>, S. Marlin<sup>2</sup>, E. Chapiro<sup>1</sup>, F. Denoyelle<sup>2</sup>, D. Sternberg<sup>3</sup>, D. Weil<sup>4</sup>, C. Petit<sup>4</sup>, E.N. Garabedian<sup>2</sup>, R. Couderc<sup>1</sup>.* 1) Dept of biochemistry and molecular biology, Hosp A.Trousseau, APHP, Paris France; 2) ORL Dept., Hosp A. Trousseau, APHP, Paris France; 3) Dept of biochemistry, Hosp Sapetriere, APHP, Paris France; 4) Unit de Gntique des Dfcits Sensoriels, CNRS URA 1968, Institut Pasteur, Paris.

The mtDNA A1555G mutation has been recognized as a main cause of sensorineural deafness in Spain families with maternally transmitted deafness. As, the A1555G mutation was also found to predispose individuals to aminoglycoside-induced hearing loss, its identification is crucial to prevent amonoglycoside-induced hearing loss. . The T7511C mutation has been previously described in only one large African American familiy with deafness. We studied 41 families and 31 sporadic cases with non-syndromic sensorineural hearing loss. In all the families, the transmission of the deafness was compatible with a maternally transmission. ARNr 12S, ARNt leucine and ARNt serine were screened by denaturing gradient gel electrophoresis and sequencing. The A1555G mutation was observed in a large family (19 affected patients) with maternally inherited deafness. The mutation was homoplasmic in all the patients screened. The affected subjects presented a congenital bilateral and sensorineural hearing loss. The deafness was severe to profound and age-stable. No other mutation was observed in the ARN 12S. In the ARNt serine, the T7511C mutation was observed in two large french families with maternally transmitted deafness. The mutation was homoplasmic or heteroplasmic. The age at onset of deafness was variable. The bilateral and sensorineural hearing loss was stable or progessive. In the ARNt leu and in the ND1gene many different variations were observed : the polymorphisms A3348G, T3396C, T3308C , T3290C and the variants G3316A, T3394C. In conclusion, A1555G and T7511C were observed in 2.6 and 4.8% of the families with maternally transmitted sensorineural deafness. This result shows that T7511C could be a frequent cause of non-syndromic sensorineural deafness and must be screened in families with maternally transmitted sensorineural deafness.



**Identification and characterization of cochlear expressed genes in the DFNB17 interval.** *J.H. Greinwald<sup>1</sup>, V. Pilipenko<sup>1</sup>, Y. Guo<sup>1</sup>, D.I. Choo<sup>1</sup>, A. Ramesh<sup>2</sup>, A.B. Giersch<sup>3</sup>, C.C. Morton<sup>3</sup>, W.C. Nichols<sup>4</sup>, R.J. Smith<sup>5</sup>.* 1) Center for Hearing and Deafness Research, Dept. of Otolaryngology, Children's Hospital Cincinnati, Cincinnati, OH; 2) Dept. of Genetics, University of Madras, Madras, India; 3) Dept. of Pathology, Brigham and Women's Hospital, Boston, MA; 4) Div. of Human Genetics, Children's Hospital Cincinnati, Cincinnati, OH; 5) Molecular Otolaryngology Research Laboratories, Dept. of Otolaryngology, University of Iowa, Iowa City, IA.

The DFNB17 locus for autosomal recessive non-syndromic hearing loss has been mapped to an approximately 3-4 cM interval on human chromosome 7q31. We have determined the cDNA sequence and genomic structure for two novel genes that map to the DFNB17 interval. The first gene, termed HMG-like gene, is composed of 12 exons and contains a putative HMG-like DNA binding domain, which presumably acts as a transcription factor. Analysis of a cochlear cDNA library revealed alternative splicing compared to other tissues. This gene is highly conserved at the DNA and protein level between mouse and human with expression noted in analysis of a mouse inner ear library. The second gene, termed nexin-like gene, contains 9 exons and has homology to the nexin family of serine proteases. The nexin-like gene is similarly expressed in human cochlear and mouse inner ear libraries, respectively. No alternative splicing was noted. *In situ* hybridization studies of the nexin-like gene revealed a unique radial pattern of semicircular canal expression in embryonic day 18 mice. Both genes are also ubiquitously expressed in many different human body tissues. To determine if mutations in either of these genes might be the cause of hearing loss at the DFNB17 locus, we screened the coding and splice-sites regions of the DFNB17 family by direct sequencing. No disease-causing mutations were found. Studies of the untranslated and promoter regions of these genes as well as two actin-related genes in the DFNB17 interval are underway.

To be presented by Karina Lezirovitz instead of R. C. Mingroni-Netto.

Program Nr: 2124

**Mitochondrial mutation A1555G (12SrRNA) and connexin 26 35delG mutation are frequent causes of deafness in Brazil.** *R.C. Mingroni-Netto<sup>1</sup>, R.S. Abreu-Silva<sup>1</sup>, M.C.C. Braga<sup>1</sup>, K. Lezirovitz<sup>1</sup>, V.A. Della-Rosa<sup>2</sup>, S. Pirana<sup>3</sup>, M. Spinelli<sup>4</sup>, P.A. Otto<sup>1</sup>.* 1) Department of Biology, Universidade de São Paulo, São Paulo, Brazil; 2) Department of Cell Biology and Genetics, Universidade Estadual de Maringá, Paraná, Brazil; 3) Department of Otorrinolaringology, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil; 4) Pontifícia Universidade Católica de São Paulo.

After detecting the mitochondrial mutation A1555G in a large Brazilian family with nine affected individuals presenting late-onset sensorineural deafness, we studied another sample of 90 individuals, ascertained through 73 index cases with deafness that attended our genetic counseling unit. All patients were tested for the presence of the mitochondrial mutation A1555G and the 35delG mutation in the Connexin 26 gene, regardless of their family history, aminoglycosides treatment, age at onset of deafness or other possible causes of deafness. 27% of the sample consisted of familial cases, the rest of patients being isolated cases. The mitochondrial mutation A1555G was detected in three index cases (4% of the total sample), two familial and one isolated case of deafness. In both the familial cases, the propositi were born to first-cousins. This mutation accounted for 2% of isolated and 10% of familial cases. The 35delG mutation in the Connexin 26 gene was detected in ten index cases (11%), seven being homozygotes and three heterozygotes for this mutation. 35delG mutation was present in 45% of familial cases and in 2% of isolated cases. Therefore, mitochondrial A1555G and Connexin 26 35delG mutations are frequent causes of hearing loss in Brazil, and together they account for 15% of all cases. The simple PCR screening for these two mutations is clearly beneficial for the diagnosis and genetic counseling of deaf patients.

Program Nr: 2125 from the 2001 ASHG Annual Meeting

**A novel mutation in Stat5b impaired DNA-binding affinity in NOD.** *A. Davoodi-Semiromi, M. Laloraya, P.G. Kumar, J-X. She.* Department of Pathology, Immunology and Laboratory Medicine, Center for Mammalian Genetics and Diabetes Center of Excellence, College of Medicine, University of Florida, Gainesville, FL 32610.

Type 1 diabetes (T1D) is a multifactorial autoimmune disease in which susceptibility is determined by genetic and environmental factors. We identified and characterized a novel mutation (C1462A) in the DNA binding domain of Stat5b in the NOD mice using mutation and functional assays. Since the T1D is tightly associated to cytokine-signaling pathways, we sequenced the Stat3, Stat5a and Stat5b genes, mapped to Idd4 interval, and identified a unique mutation that results in a L327M substitution in Stat5b of NOD. This residue is conserved in all identified mammalian STAT proteins and our data from electrophoretic mobility shift assays indicate that the mutation in NOD causes reduced DNA-binding affinity compared to wild-type animals. Our immunoblotting data also indicate that the expression levels of the perforin, Pim-1, c-Myc and IL2R-b genes are down regulated in NOD after GM-CSF stimulation in splenocytes. Identification and characterization of this defect in the Jak-Stat5b pathway provides important new insights into the immunopathogenesis of T1D. *Stat5aStat5bStat3Idd4perforinPim-1c-MycIL2R-b.*

**Identification of the first cardiac ion channel mutation in a patient with drug-induced arrhythmia provides direct evidence of genetic susceptibility to an acquired disorder.** *L. Baumbach, H. Basterrechea, E. Estrella, R. Myerburg, G. Wolff, N. Bishopric.* Pediatrics, Univ. of Miami School of Med., Miami, FL.

Long-QT syndrome (LQTS) is a disorder characterized by an abnormality in cardiac repolarization, causing syncope, seizures, and sudden death. It can be inherited or acquired as an adverse effect of drug therapy. Genes encoding cardiac ion channels have been linked to congenital LQTS, however, good genotype: phenotype correlations do not exist. Although it is not known whether acquired LQTS has a similar genetic basis, a genetic relationship between congenital and acquired forms is suspected. We are developing high-throughput rapid mutation screening methods for the three genes most frequently involved in LQTS: KVLQT1, HERG and SCN5A. Previous work has demonstrated numerous private mutations dispersed throughout these loci, making mutation detection laborious and inefficient. One of our current methodologies employs exon-specific PCR, followed by multiplex SSCP and DNA sequencing. Using this protocol, we can initially analyze all KVLQT1 exons in two SSCP gels. Similar approaches have been adapted to HERG and SCN5A. We have begun investigating genotype-phenotype correlations in congenital and acquired LQTS. Currently, 11 probands and their families are under investigation. SSCP variants in KVLQT1 have been found in two out of two patients with normal baseline QT intervals, but histories of arrhythmias exacerbated by exposures to pharmacological agents. Patient 1, has a family history of cardiac sudden death and syncope, is heterozygous for a KVLQT1 missense mutation (G189R) located in the intracellular loop between the S2 and S3 transmembrane segments. This mutation has been previously reported in three families with LQTS. We are awaiting DNA sequencing results on patient 2. **Our findings provide the first evidence for a genetic basis for pharmacologically-induced arrhythmias.** The development of high throughput mutation screening methodologies will permit genetic screening of larger patient populations, with the ultimate goal of generating a clinically available diagnostic test.

**Identification of genes from a schizophrenia susceptibility locus on 6p24 using bioinformatics and Genome mapping.** *Y. Jiang, K.S. Kendler, X. Chen, R.S. Straub, B.P. Riley.* Dept Psychiatry, Virginia Inst Psych/Behav Gen, Richmond, VA.

Schizophrenia is a psychotic disorder, characterized by a constellation of symptoms including hallucinations and delusions (psychotic symptoms) and symptoms such as severely inappropriate emotional responses, disordered thinking and concentration, erratic behavior, as well as social and occupational deterioration. The location 6p24 has previously been found to be linked with schizophrenia. Genes and genome structure present at this location have not been investigated in details. To facilitate the identification of the disease gene, a 1Mb genomic region including the positive marker D6S940 and D6S470 in previous linkage study was covered by two BAC clone contig NT\_023416 and NT\_023418 with a 50kb gap in between, which was constructed computationally using draft genomic sequence data and existing mapping data for the region. Nine BAC clones have led to the identification and relative ordering of 4 candidate genes in the region, including 1 novel transcripts, one putative gene and tandemly repeated sequences almost identical to the human retrotransposon L1. It constitutes a resource for polymorphic marker discovery and association studies to validate or reject candidate genes. Two candidate genes appear to be particularly promising based upon their proximity to the marker D6S940 and D6S470 and their likely functional roles. One putative gene predicted by Grail and Genescan involved in Apoptosis and the other gene, Matrln-3, encoding an acidic internal matrix protein with perturbation of human retrotransposon L1, a human endogenous retrovirus element in its 3' untranslated region, which is recently found important on the underlying molecular mechanism of schizophrenia.

**Prevalence of HLA-DRB1 alleles in Kuwaiti Arab children with juvenile rheumatoid arthritis.** *M.Z. Haider<sup>1</sup>, K. Alsaeid<sup>1</sup>, E.M. Ayoub<sup>2</sup>*. 1) Dept Pediatrics, Fac Medicine, Kuwait Univ, Safat, Kuwait; 2) Dept Pediatrics, Univ Florida, Gainesville, FL, USA.

The prevalence of human leukocyte antigen (HLA) DR alleles has been determined in 69 Kuwaiti Arab children with juvenile rheumatoid arthritis (JRA) and compared to that in 212 ethnically matched normal healthy controls using a PCR-sequence specific primers (PCR-SSP) method. A considerably high incidence of DR3 was detected in JRA patients compared to the controls ( $P < 0.0001$ ,  $RR = 2.235$ ). The high incidence of HLA-DR3 in JRA patients was accounted for mainly by an excess of DRB1\*0307 ( $P < 0.05$ ,  $RR = 3.072$ ) and DRB1\*0308 ( $P < 0.009$ ,  $RR = 2.663$ ) compared to the controls. Moreover, DR3 was more prevalent when patients with ANA positive JRA were analyzed separately, 73% compared to 58% in the whole JRA patients group. The frequency of DR1 was also higher in the JRA group compared to controls ( $P = 0.019$ ,  $RR = 3.585$ ). Although the incidence of some alleles was higher in the control group (DR13 and DR7), none reached a statistically significant level. All the patients with iridocyclitis had either a DR1 or DR3 allele except for one child. The frequency of DRB1\*03 was found to be much higher in polyarticular subtype of Kuwaiti JRA cases compared to the oligoarticular subgroup and the controls. Also, a nonsignificant increase in the frequency of DRB1\*04, \*11 and \*15 alleles was detected in the polyarticular subtype of the Kuwaiti JRA cases compared to the controls.

**Linkage study of reading disabilities and attention-deficit hyperactivity disorder in the chromosome 6p region.**

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It has been well documented that individuals with reading disabilities (RD) often have symptoms of attention-deficit hyperactivity disorder (ADHD). The basis for this overlap is not clear but twin studies have concluded that common genetic influences may predispose children to both conditions. We have begun to investigate the genetic overlap by genotyping markers in the region of 6p that has been reported to be linked to both the RD and ADHD phenotypes. We used two samples of small nuclear families for our studies, one ascertained through a proband with RD and the other through a proband with ADHD. We have investigated a number of markers across this region, some located in candidate genes. These include two genes in the region with the strongest support for linkage to RD, the genes for myelin oligodendrocyte glycoprotein (MOG) and the gamma aminobutyric acid (GABA) beta receptor 1 (GABAB-R1). MOG is a minor component of myelin and has been suggested to be involved in the completion and maintenance of the myelin sheath. Three possible functions for the protein have been suggested: a cellular adhesive molecule, a regulator of oligodendrocyte stability, and a mediator of interaction between myelin and the immune system. The GABAB-R1 gene is an interesting candidate gene for RD and ADHD based on the proposed role of this gene in cognition as well as the role of the GABAB receptors in modulating the release of a number of neurotransmitters including dopamine and norepinephrine, both postulated to be involved in ADHD. Thus far we have not found any evidence for linkage with these markers to either the RD or ADHD phenotypes in either sample.

**Towards identification of a susceptibility gene for bipolar affective disorder: genomic characterisation of**

**chromosome 4q35.** *I.P. Blair<sup>1</sup>, L.J. Adams<sup>1</sup>, R.F. Badenhop<sup>1</sup>, M.J. Moses<sup>1</sup>, A. Scimone<sup>1</sup>, J. Morris<sup>2</sup>, L. Ma<sup>2</sup>, C. Austin<sup>2</sup>, J.A. Donald<sup>3</sup>, P.B. Mitchell<sup>4</sup>, P.R. Schofield<sup>1</sup>.* 1) Neurobiology, Garvan Institute, Sydney, NSW, Australia; 2) Merck Laboratories, West Point, PA; 3) Dept of Biological Sciences, Macquarie University, Sydney, NSW, Australia; 4) School of Psychiatry, University of New South Wales and Prince of Wales Hospital, Sydney, NSW, Australia.

Bipolar affective disorder (BP) is characterised by aberrant mood swings resulting in alternating periods of mania and depression with reversion to otherwise normal behaviour between episodes. The etiology of BP remains unknown. Twin, family and adoption studies reveal the high heritability of BP. We recently identified a susceptibility locus for BP on chromosome 4q35 using linkage analysis in one large pedigree and suggestive linkage to 4q35 in 23 other pedigrees. Our large collection of BP pedigrees has provided us with a unique resource with which to refine the chromosome 4q35 candidate interval to a size that is amenable to positional cloning. We have used the resources of the HGP and Celera to identify overlapping sequenced BAC clones and sequence contigs that represent the region implicated in linkage analysis. A combination of bioinformatic tools and laboratory techniques have been applied to annotate this data and establish a comprehensive transcript map that spans 5 Mb and encompasses the 4q35 susceptibility locus. This map includes 10 known and 12 novel transcripts, which provide a collection of genes for investigation for association with BP. Bioinformatics, along with expression analysis, is being undertaken to give insights into the function of many of these novel genes. Transcripts that show expression and potential function in the brain are being prioritised for investigation as potential BP susceptibility genes. To date, 7 genes have been screened for SNPs in cases and controls from 7 pedigrees which support 4q35 linkage. Numerous intragenic SNPs have been identified and phase known SNP haplotypes are being examined for association with BP in a much larger case-control cohort. This strategy is being applied to all candidate genes from the region.



**Transcriptional dysregulation in Huntington's disease.** *J. Duce*<sup>1</sup>, *C. Hartog*<sup>1</sup>, *L. Elliston*<sup>1</sup>, *G. Bates*<sup>2</sup>, *L. Jones*<sup>1</sup>. 1) Institute of Medical Genetics, Univ of Wales Col Medicine, Cardiff, UK; 2) Division of Medical and Molecular Genetics, GKT School of Medicine, 8th Floor Guy's Tower, Guy's Hospital, London SE1 9RT, UK.

Huntington's disease (HD) is an autosomal dominant neurodegeneration associated with an expanded polyglutamine tract close to the N-terminus of the protein huntingtin. The mechanism by which long glutamine tracts cause HD remains unclear, and examination of huntingtin interactions may provide clues to the pathogenic mechanism. Various transcriptional regulatory proteins interact with huntingtin and we have examined the interactions between huntingtin, the nuclear receptor co-repressor (N-CoR) and the silencing mediator of retinoic acid and thyroid receptor (SMRT), related proteins which repress transcription through thyroid hormone and retinoic acid receptors as well as a number of other sequence-specific DNA binding proteins. Yeast two-hybrid experiments reveal that the N-CoR-huntingtin interaction is stronger than the SMRT-huntingtin interaction, but in both cases interaction is mediated through the homologous C-terminal repression domains. An artificial GAL4-mediated transcriptional reporter system shows that N-terminal huntingtin can recruit repressive activity, and immunohistochemistry in HD brains reveals a marked relocalisation of both N-CoR and SMRT in both early and late stages of disease. Both proteins are recruited infrequently into intranuclear inclusions, but demonstrate a more general relocalisation within neurones, particularly in cortical layers V and VI, and also give consistently stronger immunoreactive signals in HD than control brain. To further examine the effect huntingtin may be having on global gene expression we have examined the levels of gene expression in R6/2 mice, transgenic for exon 1 of the huntingtin protein with around 150 glutamines, using the Affymetrix U74A Genechip. Dysregulated gene expression mediated through the interactions described above may contribute to the alterations in gene expression seen in HD brain and thus contribute to the pathogenesis of HD.

**Chromosome 15 cryptic rearrangements in pervasive developmental disorders.** *F. Gurrieri, L. Russo, T. Giordano, E. De Vincenzi, G. Neri.* *Genetica Medica, Univ Cattolica, Rome, Italy.*

Pervasive developmental disorders (PDDs) represent an heterogeneous group of behavioural deficits, including autism and atypical autism, characterized by impaired communication and social interaction, restricted interests and stereotyped behaviours. PDDs affect around 1:2.500 individuals within the first three years of life with a sex ratio M:F = 4:1. A strong genetic basis has been recognized and several genomic studies, as well as cytogenetic observations, have identified at least 12 candidate loci for genes predisposing to PDD. The 15q11-q13 genomic region is most interesting because linkage/association studies have repeatedly shown positive lodscores in multiplex PDD families. In addition, cytogenetic rearrangements, such as inv-dup15 or cryptic interstitial duplications, have been non-randomly associated with atypical autism. In order to define the prevalence of cryptic 15q11-q13 duplications in PDD, we have genotyped 100 patients with their normal parents by using a set of microsatellite markers mapped in this critical region. The patients were selected through a collaboration with various child neuropsychiatry centers. The molecular screening of 100 PDD families allowed the identification of a patient carrying a molecular duplication of the critical interval including markers from D15S817 to D15S113, which was clearly pathogenic [Gurrieri et al.: *Neurology* 52:1694-97, 1999]. In addition, a maternally derived duplication at a single locus, D15S817, was identified in another two unrelated PDD patients. The presence of such duplication was confirmed with different sets of nested primers. We could not identify any D15S817 duplication in 200 control chromosomes. Although the pathogenicity of these rearrangements needs to be confirmed by further studies, our results show that a cryptic 15q11-q13 duplication is responsible for PDD in 1-3% of patients with this diagnosis. This percentage is relatively high, considering the marked genetic heterogeneity of PDD. Supported by grants from MURST and Sigma-tau.

**DIBD1, a novel gene homologous to *Saccaromyces cerevisiae* ALG9, is disrupted in a family with bipolar affective disorder by a translocation breakpoint at 11q23.** *B.E. Baysal*<sup>1,3</sup>, *J.E. Willett-Brozick*<sup>1</sup>, *J.A. Badner*<sup>2</sup>, *R.E. Ferrell*<sup>3</sup>, *V.A. Nimgaonkar*<sup>1</sup>, *S. Detera-Wadleigh*<sup>4</sup>. 1) Dept. Psychiatry Univ. Pittsburgh Med Ctr; 2) Dept. Psychiatry Univ. Chicago; 3) Dept Hum Genet Univ. Pittsburgh; 4) NIMH Intramural Research Program, NIH.

Bipolar affective disorder (BPAD) is a complex neuropsychiatric disorder characterized by extreme mood swings. We previously described a pedigree in which five individuals with BPAD and one individual with recurrent major depression were carriers of a reciprocal chromosomal translocation t(9;11)(p24;q23). Gene content analyses of breakpoint junctions revealed disruption of a novel gene (DIBD1) at 11q23, a region that has also been implicated in the etiology of schizophrenia and Tourette syndrome. DIBD1 is predicted to encode a 611 amino acid long transmembranous protein, homologous to the *Saccaromyces cerevisiae* ALG9, and is composed of 15 exons spanning approximately 85 kb. ALG9 encodes a mannosyltransferase involved in the protein N-glycosylation pathway, the inborn errors of which cause congenital disorders of glycosylation in human. DIBD1 is ubiquitously expressed in a variety of tissues, including all tested subregions of the brain. Sequence analysis revealed three intra-genic SNPs. The valine residue in the non-synonymous SNP V289I is conserved in 10 other multicellular eukaryotic species, whereas its frequency is approximately 65% in human. We performed linkage and transmission disequilibrium test (TDT) analyses by genotyping 950 individuals including 316 affecteds from two bipolar pedigree series using four tightly linked STRPs and V289I. Nominally significant results were obtained in the subset of 22 multiplex pedigrees under a broad phenotypic definition. Linkage analysis with an intronic STRP gave a lod score of 1.18, ( $p=0.0098$ ) and the TDT using a two-marker-haplotype was significant at  $p=0.04$ . Thus, DIBD1 is a novel gene disrupted in a family with BPAD and the identification of marginally significant linkage/TDT results and the presence of a common non-synonymous SNP at an evolutionarily conserved amino acid position may suggest a role for it in disease pathogenesis in the general BPAD patient population.

**Association of the calcium-sensing receptor gene in Alzheimer's disease susceptibility.** *Y.P. Conley<sup>1,2</sup>, D.N. Finegold<sup>2</sup>, R.E. Ferrell<sup>2</sup>*. 1) Health Promotion & Development, Univ Pittsburgh, Pittsburgh, PA; 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 have confirmed APP and ApoE as alternative ligands for the CASR, which demonstrates that the CASR could have a function other than mineral ion homeostasis in the brain. Given the association of APP and ApoE with Alzheimer's disease we conducted an age-matched case control study using a polymorphic dinucleotide marker found in intron 4 of the CASR. 284 Alzheimer's disease patients and 97 age-matched non-demented control samples were genotyped for the intragenic marker. We find that the frequency of several alleles differ significantly between AD patients and controls and when the data is dichotomized based on allele frequencies,  $\leq 228$ bp and  $>228$ bp the AD patients have significantly shorter repeat lengths (34%) compared to the controls (19%) with an OR of 2.1 ( $p < 0.005$ ). When the data is dichotomized based on presence of an allele  $\geq 230$  bp in a genotype, 82% of the AD patients had genotypes containing an allele  $\geq 230$  bp compared with 96% of the controls with an OR of 5.1 ( $p < 0.005$ ). Activation of the CASR ultimately results in an increase in intracellular calcium ions, providing a mechanism by which APP and ApoE increase intracellular calcium ion concentration and suggests a mechanism by which they exert their neurotoxic effects. Whether this length polymorphism is functional, or is a marker for functional variation elsewhere in the CASR gene influencing variation in AD susceptibility, this case/control study confirms association of the CASR gene in AD status and strengthens our hypothesis that it plays a role in the Alzheimer's disease process.

**The Genetic Architecture of Exploratory and Fear like behavior in mice: A comparison of two complementary strategies for fine mapping QTLs.** *H. Gershenfeld<sup>1</sup>, Y. Lou<sup>1</sup>, M. Anyango<sup>2</sup>, N. Mohibullah<sup>1</sup>, A. Osoti<sup>2</sup>, R. King<sup>2</sup>, F. Iraqi<sup>2</sup>.* 1) Dept Psychiatry, UTSW, Dallas, TX; 2) ILRI, Nairobi, Kenya.

**BACKGROUND:** Prior work with F2 and N2 crosses have defined loci on chr 1, 10, and 19 that influence open field (OF) and Light-Dark (LD) behaviors by quantitative trait loci (QTL) mapping in mice. **AIMS:** To compare two methods of localizing these QTL and to define candidate regions for human studies. **METHODS:** Interval Specific Congenic Strains (ISCS) for the distal region of chr 10 containing the Exq QTL were bred and studied. As a second strategy, F12 Advanced Intercross Line (AIL) mice (N=1130), originally derived from crossing two markedly differing, parental strains A and C57BL/6 mice were sequentially phenotyped, genotyped, and QTL mapped. **RESULTS:** The ICSC data suggest that the Exq locus is either composed of two linked loci on distal chr. 10 or the possibility of a telomeric location beyond the available markers. From the F12 AIL data, the Exq locus mapped robustly with high statistical significance and explained 4% of the total phenotypic variance for LD behavior. Surprisingly, the large regions containing OF loci on chr. 1 and 19 were localized to several regions with weakly suggestive LOD scores, each explaining about 1% of the variance. **CONCLUSIONS:** These results suggest that gene interactions between linked loci may explain some of the non-replications and difficulties of genome mapping in psychiatric illness. Three findings may be generalizable to complex trait mapping, namely that 1) behavior results from inheriting "many small things" (i.e., modest effect loci explaining 1-5% of the variance), 2) the congenic data suggest gene interactions exist where only combinations of loci have detectable effects, and 3) the genetic architecture underlying complex traits becomes the critical determinant of fine mapping success, highlighting the difference of loci acting in isolation in congenic strains versus the F12 AIL where the Exq loci work in the context of other B6 loci in the genetic background. Support: NIH Grant RO1 MH58882 (HG) , The Southwestern Medical Foundation (HG).

***Noggin* is not a major gene for neural tube defects or Chiari type 1 malformation.** K. Bauer<sup>1</sup>, E.C. Melvin<sup>1</sup>, L. Mehlretter<sup>1</sup>, C. Drake<sup>1</sup>, T.M. George<sup>1</sup>, D. Enterline<sup>1</sup>, M. Hauser<sup>1</sup>, T.H. Milhorat<sup>3</sup>, J.S. Nye<sup>2</sup> and NTD Collaborative Group. 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Northwestern University Medical Center, Chicago, IL; 3) State University of New York, Brooklyn, NY.

*Noggin* encodes a bone morphogenetic protein (BMP) antagonist expressed in the developing neural tube, condensing cartilage, and chondrocytes. *Noggin* knockout mice show various defects affecting the somites, limbs and neural tube. We investigated *Noggin* using denaturing high performance liquid chromatography (DHPLC) as a candidate gene for neural tube defects (NTDs) and Chiari type 1 malformation. The screen included 202 cases with NTD (142 simplex cases and 60 multiplex cases; 143 lumbosacral myelomeningocele {MM}, 12 lumbosacral/thoracic MM, 23 thoracic MM 12 lipomyelomeningoceles, and 22 miscellaneous NTDs) and 33 individuals diagnosed with non-syndromic Chiari type 1 malformation. The region screened included the 699bp of coding region, 365bp of the 5 prime untranslated region (UTR) and 17 bp of the 3 prime UTR. The DNA from cases was grouped into 42 pools and screened utilizing denaturing high performance liquid chromatography (DHPLC). Any pool showing a variant was separated into its individual constituents and further studied. One heterozygous variant was identified in a male patient with a lumbosacral myelomeningocele. The individual and his available family members were sequenced. The patient's father and a female sibling also carried the variant allele, but neither is affected with open NTD; the patient's mother and another sibling do not have the variant allele. The C->G sequence variant occurred at position 1064, yielding an amino acid residue change from proline to histidine. The variant found in the NTD patient is a new variant. This sequence variation may be unrelated to the patient's NTD since the variant is also present in unaffected relatives, but further investigation is underway to exclude compound heterozygosity in the affected patient. Based on these results, *Noggin* is unlikely to be a major gene predisposing to NTDs or Chiari type 1 malformation.

**Estrogen Receptor gene polymorphism in Italian patients with multiple sclerosis.** *R. Cittadella<sup>1</sup>, I. Manna<sup>1</sup>, V. Andreoli<sup>1</sup>, A. La Russa<sup>1</sup>, G. La Porta<sup>1</sup>, L. Cresibene<sup>1</sup>, L. Bastone<sup>1</sup>, R. Nisticò<sup>2</sup>, L. Mangone<sup>2</sup>, N. Romeo<sup>1</sup>, M. Caracciolo<sup>1</sup>, P.A. Serra<sup>1</sup>, A. Quattrone<sup>1,2</sup>.* 1) IMSEB-National Research Council, Cosenza, Italy; 2) Institute of Neurology, University Magna Graecia, Catanzaro, Italy.

Development of multiple sclerosis (MS), an autoimmune disease, is believed to involve genetic as well as environmental factors. The estrogen receptor 1 gene (ESR1) is an interesting candidate to uncover the genetic background of MS, because the expression of ESR1 has been identified in cells of the immune system and, thus genetic variability of ESR1 may play a role in the development of MS. Estrogen has been reported to have immunosuppressive functions, and most of its effects are mediated via receptors whose function and expression may be modified by DNA polymorphisms. In the present study we investigated the possible role of ESR1 in the pathogenesis of MS by studying intronic polymorphisms of the ESR1 gene. In its first intron two polymorphisms have been shown: T to C approximately 400 bp upstream from exon-2 and A to G approximately 350 bp. These polymorphisms were determined by restriction fragment length polymorphism (RFLP) using PvuII and XbaI respectively after polymerase chain reaction (PCR). One-hundred and thirty-two unrelated Italian patients with clinically definite MS (81 females and 51 males) and 129 unrelated healthy controls (66 females and 63 males), with a similar age range, were selected for this study; all the case patients and the controls were from the same geographical area (Calabria region, Southern Italy). The distribution of the genotypes was very similar to that previously reported in the Caucasian population, but there was no significant difference in allele or genotype frequencies between patients and controls. In conclusion, our findings suggest that the ESR1 gene does not contribute to the development of MS.

**Dopamine D4 receptor (DRD4) and intensity of reaction trait during infancy.** A. De Luca<sup>1,2</sup>, M. Rizzardi<sup>3</sup>, A. Buccino<sup>1,2</sup>, I. Torrente<sup>1,2</sup>, M. Mangino<sup>1,2</sup>, G.P. Salvioli<sup>4</sup>, R. Alessandrini<sup>4</sup>, B. Dallapiccola<sup>1,5</sup>, G. Novelli<sup>2</sup>. 1) Ist. CSS-Mendel, Rome, Italy; 2) Dip. di Biopatologia e Diagnostica per Immagini, Univ. di Roma "Tor Vergata", Rome, Italy; 3) Fac. di Scienze della Formazione, Univ. di Urbino, Urbino, Italy; 4) Ist. di Ped. Preventiva e Neonatologia, Bologna, Italy; 5) Dip. di Medicina Sperimentale e Patologia, Univ. di Roma "La Sapienza", Rome, Italy.

Temperament dimensions are developmental phenomena affected by genetic and environmental factors. During development, some behaviours can either appear or disappear, while others remain continuous and, during the course of development, become more differentiated. We had previously examined the relationship between the common dopamine D4 receptor (DRD4) exon III repeat polymorphism and infants behaviour in Italian neonates at 1 and 5 months of life (De Luca et al. *Neurogenetics* 2001;3:79-82). We found a significant correlation between adaptability and DRD4 long alleles of exon III repeat. In order to extend preliminary results, we longitudinally followed the same group of infants up to 3 years, using the Toddler Temperament Scale (Fullard W., McDevitt S.C. and Carey W.B. 1979). We detected a significant association between intensity of reaction and DRD4 genotypes (4/7 and 4/4). Infants with 4/7 genotype had significantly lower scores on intensity of reaction than infants with 4/4 genotypes [t-value=2.9; df 63; p<0.01]. Infants with 4/7 genotype had significantly lower scores [mean=3.5±0.83, N=18] on intensity of reaction than infants with 4/4 genotypes [mean=4.1±0.79, N=47]. Similar results were obtained either when genotypes were classified by the presence or the absence of the 7-repeat allele, or by the long (>6 repeats) versus the short (<5 repeats) DRD4 alleles. These results confirm and extend the genetic influence of DRD4 gene in human temperament during development.



**Developing of full-length cDNA for novel genes expressed in brain from chromosome 18q21 to create a gene panel for variation/mutation screen in bipolar disorder.** *H. Chen*<sup>1</sup>, *C.A. Ross*<sup>1, 2</sup>, *N. Wang*<sup>1</sup>, *Y. Huo*<sup>1</sup>, *P. Sklar*<sup>3</sup>, *D.F. MacKinnon*<sup>1</sup>, *J. Potash*<sup>1</sup>, *S.G. Simpson*<sup>1</sup>, *J. Chen*<sup>4</sup>, *S. Wang*<sup>4</sup>, *F.J. McMahon*<sup>5</sup>, *J.R. DePaulo, Jr.*<sup>1</sup>, *M.G. McInnis*<sup>1</sup>. 1) Department of Psychiatry and Behavioral Sciences; 2) Department of Neuroscience, Johns Hopkins University, Baltimore, MD; 3) Massachusetts General Hospital, Boston, MA; 4) Department of Medicine; 5) Department of Psychiatry, University of Chicago, Chicago, IL.

A bipolar disorder susceptibility locus has been linked to a region on human chromosome 18q21 (Stine et al, 1995; McMahon et al, 1997). To search for candidate genes potentially involved in the disorder, exon trapping was performed on cosmids isolated from 18q21, and resulted in the identification of 145 unique exons, of which 22 are identical to known genes and ESTs, 24 to ESTs only, and 101 have no homology to known genes. A strategy was developed for the generation of a panel of full-length cDNAs corresponding to the identified exons for variation/mutation studies of novel genes expressed in brain in bipolar disorder. This strategy consists of using a modified GLGI method (Generation of Longer 3' cDNA from SAGE tags for Gene Identification) to generate 3' cDNA, and 5' RACE method to generate 5' cDNA, and of combining 3' and 5' cDNAs into full-length cDNA corresponding to each gene expressed in brain. Northern blot analysis will be carried out to examine the expression patterns and the sizes of mRNA species of each gene in the panel. Among 28 trapped exons tested that have no homology to known genes and ESTs, 8 showed the amplification of 0.5-1.6 kb of 3' cDNAs. The full-length cDNAs for two novel genes, a NEDD4-like ubiquitin-protein ligase (termed NEDD4L) and a novel zinc finger gene, have been generated through combining their 3' and 5' cDNA sequences. The remaining 93 trapped exons (excluding the 22 exons identical to known genes) are currently being tested according to the above procedures. The establishment of full-length cDNA panel for genes in 18q21 expressed in brain will be prioritized for variation/mutation screen in bipolar subjects.

**Possible association of NTDs with a poly-histidine tract polymorphism in the ZIC2 gene.** *L.Y. Brown<sup>1</sup>, S.E.*

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Neural tube defects (NTDs) and brain malformations represent a common finding in chromosome 13q deletion patients. These defects may be due, in part, to hemizyosity for ZIC2, which is located in the 13q32 critical deletion region. Heterozygous mutation in ZIC2 result in holoprosencephaly (HPE) in humans, and diminished expression of Zic2 results in HPE as well as lumbo-sacral NTDs in mice. Taken together, these observations led us to hypothesize that ZIC2 mutations may be a cause of isolated NTD. To test this, we screened 192 NTD patients for mutations in ZIC2. We did not find ZIC2 mutations in these patients; however, we did find evidence of an association between a histidine tract polymorphism in ZIC2 and NTDs.

The ZIC2 gene normally contains a tract of 9 histidines coded for by a triplet repeat. Occasionally this repeat is expanded to code for 10 histidines (10H). While only 0.5% of normal controls carry the (10H) variant of the ZIC2 gene, 6% of NTD patients were found to be heterozygous for the 10H allele ( $c^2 = 9.03$ , significant at the .005 level). When we considered only Hispanic NTD patients and controls, 25% of NTD patients were found to be heterozygous (9H/10H) compared to only 1% of controls ( $c^2 = 17$ ). We attempted to confirm this result with a transmission disequilibrium test (TDT), but our sample was too small to reach definitive conclusions. If this association is confirmed in a larger sample, then subtle alterations in ZIC2 activity may confer a risk of NTD.

**Amyloid b-secretase gene (BACE) is neither mutated nor associated with early-onset Alzheimers disease.** *M. Cruts<sup>1</sup>, B. Dermaut<sup>1</sup>, R. Rademakers<sup>1</sup>, G. Roks<sup>2</sup>, M. Van den Broeck<sup>1</sup>, G. Munteanu<sup>1</sup>, C.M. van Duijn<sup>1,2</sup>, C. Van Broeckhoven<sup>1</sup>.* 1) Flanders Interuniversity Institute for Biotechnology (VIB), University of Antwerp (U.I.A.), Department of Biochemistry, Antwerpen, Belgium; 2) Department of Epidemiology and Biostatistics, Erasmus Medical Centre, Rotterdam, The Netherlands.

The major hallmark of Alzheimers disease (AD) pathology is b-amyloid (Ab) deposition in the brain. Three protease activities named a-, b- and g-secretase are crucial in the proteolytic cleavage of the amyloid precursor protein (APP) to generate Ab. Recently, the b-secretase was identified and named beta-site APP-cleaving enzyme (BACE). Since all known familial early-onset AD (EOAD) related mutations in APP and presenilin (PSEN) increase Ab production by affecting one of the secretase activities, we hypothesized that genetic variations within BACE might be similarly involved in the etiology of EOAD. We performed genetic linkage analyses in families of 9 autosomal dominant probands that had no mutation in APP or PSEN using markers D11S1327, D11S939, D11S1336 located near BACE. No family showed conclusive linkage and in 3 families linkage with BACE was excluded. cDNA and genomic mutation analysis revealed no mutations except for a frequent silent c.786C>G polymorphism (V262) and a c.840+5G>T variation at the fifth nucleotide of intron 5 that was observed in only 1 patient. Next, the V262 polymorphism was analyzed in a sample of 101 presenile AD patients and 185 control subjects. Allele and genotype distributions were not significantly different in cases and controls. Also, no differences were found when the sample was stratified for positive family history or the presence of an APOE4 allele. Together these results show that BACE is not genetically involved in the etiology of EOAD.

**Association study between CAG trinucleotide repeats in the PCQAP gene (PC2 glutamine/Q-rich-associated protein) of the DiGeorge chromosomal region and schizophrenia.** *E. Conti*<sup>1,2</sup>, *A. De Luca*<sup>1,2</sup>, *F. Amati*<sup>2</sup>, *A. Pasini*<sup>3</sup>, *G. Spalletta*<sup>3,4</sup>, *L. Berti*<sup>5</sup>, *G. Mittler*<sup>5</sup>, *N. Grifone*<sup>1</sup>, *B. Dallapiccola*<sup>1,6</sup>, *M. Meisterernst*<sup>5</sup>, *G. Novelli*<sup>2</sup>. 1) Ist. CSS-Mendel, Rome, RM, Italy; 2) Dip. di Biopatologia e Diagnostica per Immagini, Univ. di Roma "Tor Vergata", Rome, Italy; 3) Dip. Di Psichiatria, Univ. di Roma "Tor Vergata", Rome, Italy; 4) IRCCS Santa Lucia, Rome, Italy; 5) Department of Proteinbiochemistry, Institute of Molecular Immunology-GSF, Munich, Germany; 6) Dip. di Medicina Sperimentale e Patologia, Univ. di Roma "La Sapienza", Rome, Italy.

Reports showing anticipation in families with schizophrenia suggested that a trinucleotide repeat expansion mechanism may be involved in the pathogenesis of the disease. Furthermore, studies of genome-wide trinucleotide repeats using the repeat expansion detection technique suggested possible association of CAG/CTG repeat tracts with schizophrenia. Increased incidence of schizophrenia or schizoaffective disorders has been reported in patients affected by DiGeorge/Velo-Cardio-Facial syndrome (DGS/VCFS). We evaluated the potential role of a CAG polymorphic stretch in the coding region of the novel PCQAP gene mapping within the DiGeorge chromosomal region in schizophrenia susceptibility. We studied the distribution of the CAG repeat alleles in 102 schizophrenic patients and in 100 healthy controls. No unusual expanded CAG trinucleotide repeat was detected in the schizophrenic compared to control subjects. However, an analysis of the difference of allele sizes revealed a significantly greater number of patients with schizophrenia having longer allele sizes (> 17 repeats) when compared to normal controls [O.R.=2.12; 1.15<OR<3.94; CI 95%;  $\chi^2=6.72$ ;  $P<0.01$ ]. This finding may be of functional significance as the PCQAP protein is a component of a multiprotein complex involved in transcription regulation. Large difference in allele sizes could alter the complex stoichiometry resulting in perturbation of gene expression during embryonic development.

**The role for NMDA receptors in the pathogenesis of schizophrenia: no evidence that the NMDAR2B gene is associated with susceptibility to schizophrenia.** *R. Gulli<sup>1</sup>, R. Pioli<sup>2</sup>, A. De Luca<sup>3,4</sup>, S. Begni<sup>2</sup>, A. Pasini<sup>4</sup>, G. Spalletta<sup>4</sup>, E. Bellone<sup>1</sup>, A. Pizzuti<sup>3,5</sup>, G. Novelli<sup>4</sup>, B. Dallapiccola<sup>3,5</sup>, F. Ajmar<sup>1</sup>, M. Gennarelli<sup>2</sup>, P. Mandich<sup>1</sup>, E. Di Maria<sup>1</sup>.* 1) University of Genova, Italy; 2) IRCCS Fatebenefratelli, Brescia, Italy; 3) C.S.S., Mendel Institute Rome, Italy; 4) Tor Vergata University of Rome, Italy; 5) La Sapienza University of Rome, Italy.

The N-methyl-D-aspartate receptors (NMDARs) hypofunction as a mechanism leading to the development of schizophrenia was raised by the evidences that administration of non-competitive NMDAR antagonists trigger psychotic symptoms in schizophrenic patients, and induce psychotic-like behaviours in normal subjects. Animal models further supported this hypothesis. Functional NMDARs comprises the NR1 subunit and one of four NR2 subunits. The NR2B gene, named GRIN2B, is expressed in human brains in the hippocampus, basal ganglia and cerebral cortex. The hypothesis that variations within the genetic background of the NMDAR2B are associated with susceptibility to schizophrenia was investigated through a case-control study. A panel of nine polymorphisms (allele frequencies > 1%) was identified through "in silico" analysis and direct sequencing of a sample series. Allele and genotype frequencies were estimated for five informative polymorphisms on a series of 160 unrelated patients with schizophrenia, assessed according with DSM-IV criteria, and compared with normal subjects. A preliminary analysis on a subset of patients has showed a difference in allele frequencies of one SNP between patients and controls. Conversely, the case-control study on the whole series failed to reveal any significant difference in allele and genotype frequencies. The analysis on extended haplotypes confirmed this finding. This study did not provide any evidence that the NMDAR2B genetic background plays a major role in the development of schizophrenia. However, since no functional variant (i.e. coding SNPs) was detected in the coding sequence, the gene is supposed to be highly conserved. A possible role for the GRIN2B gene in schizophrenia could be eventually sustained by functional variations related with its expression. (Partially granted by Min.Sanita' to EDM).

**Screening of candidate genes for schizophrenia susceptibility in chromosome 8p12-p21.** *E.A. Donarum<sup>1</sup>, K.M. Brown<sup>1</sup>, J.M. Devaney<sup>1</sup>, G. Stevenson<sup>1</sup>, D.A. Stephan<sup>1</sup>, A.E. Pulver<sup>2</sup>.* 1) Research Center for Genetic Medicine, Childrens National Medical Center, Washington, D.C; 2) Johns Hopkins University School of Medicine, Epidemiology-Genetics Program in Psychiatry, Baltimore, MD.

Evidence from several schizophrenia linkage studies, including two genome scans (Blouin et al., 1998; Gurling et al., 2001), implicate chromosomal region 8p21 in schizophrenia susceptibility. From our sample of 54 multiplex schizophrenia pedigrees exhibiting suggestive linkage evidence in the region, we have selected probands from 1.) families exhibiting allele sharing amongst affected family members for markers in the 8p12-p21 region (over 20 cM), and 2.) genome scan families with schizophrenia spectrum disorders diagnosed in relatives ( see Pulver et al., 2000, for stratification analyses of chromosome 8 based on diagnoses in first degree relatives). Genes were chosen as candidates based upon their physical location and their function or expression patterns relevant to schizophrenia hypotheses. The positional candidates included adrenergic, cholinergic, and nictotinic receptors, among others. Mutation detection techniques including from SSCP, dHPLC, and PTT were used to analyse of DNA from 11 probands for candidate genes including: ADRA1A, ADRA1C, NEFL, NEF3, GABRR2, GABRR1, LPL, PNOC, CLU, ADRB3-1,ADRB3-2, GFRA2-1 GFRA2-2, SLC18A1, CHRN3, STC1, and CHRNA2. Aberrant SSCP bands/ (heteroduplexes) were then sequenced. No meaningful amino acid changes were found, excluding these candidates for schizophrenia liability in these carefully selected probands.

**Variance in age of onset of Huntington's disease that is independent of CAG repeat length remains highly heritable.** *J.M. Andresen<sup>1</sup>, L.R. Cardon<sup>2</sup>, S.S. Cherny<sup>2</sup>, J.F. Gusella<sup>3</sup>, M.E. MacDonald<sup>3</sup>, D.E. Housman<sup>1</sup>, N.S. Wexler<sup>4</sup>.* 1) MIT, Cambridge, MA; 2) University of Oxford, United Kingdom; 3) Massachusetts General Hospital, Boston, MA; 4) Columbia University, New York, NY.

Huntington's disease (HD) is a fatal, autosomal dominant neurological illness which causes involuntary movements, severe emotional disturbance, and cognitive decline. The mutation responsible for HD is the expansion of an unstable CAG repeat embedded in the translated open reading frame of exon 1 of the huntingtin gene, which causes expression of an expanded polyglutamine repeat in the huntingtin protein. Huntingtin alleles with 40 or more CAG repeats are 100% penetrant within a normal human lifespan, though age of onset and clinical presentation of the disease vary dramatically among individuals. The length of the triplet CAG repeat is a major determinant of age of onset for HD, with age of onset varying inversely with the number of CAG repeats in the gene. In a large Venezuelan HD kindred we have characterized, repeat length is responsible for 72% of the variance in age of onset. The variance in age of onset after repeat length is factored out, however, remains highly heritable. A residualized age-of-onset quantitative trait score was created which removed that portion of age of onset that could be explained by CAG repeat length. Using maximum-likelihood variance components to estimate between and within sib-pair variances on this new phenotype, the sibling intraclass correlation on residualized age-of-onset was calculated for 172 sibling relationships of 337 individuals who had between 40 and 50 repeats. The correlation was estimated at 0.39 ( $p < 0.0001$ ), which corresponds to a heritability of 78%, assuming shared environmental influences are negligible. This analysis justifies a genome-wide scan for linkage in the Venezuelan HD Kindred to identify quantitative trait loci contributing to the determination of age of onset in this population. The eventual identification of polymorphisms that modify age of onset for HD should give new insight into the pathological processes that underlie HD and could materially assist the development of strategies for clinical intervention.

**PICK1: A SERINE RACEMASE INTERACTOR, WHICH IS LOCATED ON CHROMOSOME 22Q11-13.** *K. Fujii<sup>1</sup>, Y. Ozeki<sup>1</sup>, A. Kamiya<sup>1</sup>, H. Otsuki<sup>1</sup>, N. Yamada<sup>1</sup>, M. Ohkawa<sup>1</sup>, A. Sawa<sup>2</sup>.* 1) Psychiatry, Shiga Univ.of Med.Sci., Otsu, Shiga, Japan; 2) Neuroscience and Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, Maryland, USA.

Schizophrenia is a common mental disorder which occurs in approximately 1 out of 100 births; however, its pathogenesis still remains elusive. From clinical pharmacological evidence, a deficit of glutaminergic neurotransmission, especially via the NMDA receptor, has been highly suggested. D-serine can activate the NMDA receptor, acting on the glycine site, as an endogenous co-agonist of glutamate. Clinical trials of D-serine with classical neuroleptics can improve part of the symptoms in schizophrenic patients, suggesting a role of D-serine in schizophrenia. Serine racemase, an enzyme enriched in astrocytes, can produce D-serine. To clarify its regulatory mechanism, we have carried out yeast-2-hybrid screening, using the full length of human serine racemase as the bait. We have analyzed an adult human brain library, and picked up 2 positive clones. Both of them represented PICK1. The gene for PICK1 is located on chromosome 22q11-13, which has been suggested as a genetic locus for schizophrenia.



**Transcriptome analysis in two transgenic mouse models for Huntington's Disease.** *S.E. Kotliarova<sup>1</sup>, C. Uchikawa<sup>1</sup>, Y.V. Kotliarov<sup>2</sup>, Y. Kamide<sup>1</sup>, F. Oyama<sup>3</sup>, N. Nukina<sup>1</sup>.* 1) Lab. for CAG repeat diseases, Brain Science Institute, RIKEN, Wako-shi, Saitama, Japan; 2) Research into Artifacts, Center for Engineering, University of Tokyo; 3) Department of Neuropathology, Graduate School of Medicine, University of Tokyo.

Huntington's disease is a hereditary neurodegenerative disorder caused by expansion of the CAG repeat (translated as polyglutamine (pQ) tract) in the exon 1 (ex1) of the huntingtin (htt) gene. We used the construct of human ex1 with expanded pQ, fused with EGFP to create the HD150-1 transgenic mouse model for HD. Although mice expressing 150-180 pQ revealed progressive neurological phenotype, neuronal cell loss was not observed in HD150-1 mice suggesting neuronal dysfunction is responsible for disease phenotype.

To find genes affected by mutant htt, we applied GeneChip technology (Affymetrix, Mu11K) for the analysis of mRNA from brain of HD150-1 mice in asymptomatic animals at 8 weeks and in early symptomatic animals at 12 weeks. In addition, we used R6/2 8week mice to find the commonly affected genes in 2 models. To analyze data we developed the software for Multiple Comparisons Analysis (MCA) using VBA on MSExcel. As a result we found differential expression of about 360 genes (from examined ~11000) in transgenic mice and their control littermates in all animal groups. We annotated 78% of potentially affected genes (the rest 22% were ESTs). Among the known genes 22.7% belong to signal transduction pathways; 13.5% to basic anabolism and catabolism pathways; 12.8% involved in nervous system function; 12.1% in immunity; 11% in RNA synthesis and processing; 8.9% in protein synthesis and modification and 7.8% in cell structure/motility. The majority of differentially expressed genes (57.5%) were down regulated.

We analyzed expression of genes involved in nervous system function in more details by TaqMan-based RT-PCR assay. Such genes as enkephalin and somatostatin are affected in different HD mouse models as well as in other triplet diseases mouse models, suggesting that the common mechanism may underlie neuronal dysfunction in CAG repeat diseases.

**Huntington's disease intermediate allele and new variant CJD.** *P.S. Harper<sup>1</sup>, R. Evans<sup>2</sup>, L. Elliston<sup>1</sup>, J.W. Ironside<sup>2</sup>, A.L. Jones<sup>1</sup>, L. Lazarou<sup>1</sup>.* 1) Wales Medical Genetics Service and Inst Medical Genetics, Univ of Wales Col Medicine, Cardiff, Wales, UK; 2) National CJD Surveillance Unit, Western General Hospital, Edinburgh, UK.

We report a female patient with onset age 28 and death age 30 years from a rapidly progressive neurodegenerative illness, with an initial psychiatric presentation but including involuntary movements, raising a clinical suspicion of Huntington's disease (HD). The patient's mother had also died from a poorly defined dementing illness age 67 years, but there was no family history of HD. Molecular analysis of the daughter showed an expanded HD allele of 36 repeats, but this was not considered an adequate cause for the disorder at this young age.

Neuropathology showed a spongiform encephalopathy with amyloid plaques and undefined deposition of prion protein, the features being characteristic of new variant Creutzfeldt-Jakob disease (nvCJD), associated in the UK with the infective prion agent of bovine spongiform encephalopathy. There were no brain features of HD.

This unusual case raises the possibility that the HD locus might influence nvCJD susceptibility, or that prion exposure might modify expression of an HD intermediate allele. Investigation of a full series of nvCJD and classical CJD with respect to the HD and other CAG repeats is required to determine whether the present case represents more than a chance association.

**Study on the genes responsible for E2F-1-mediated neuronal death in Down syndrome brains.** *K. Motonaga<sup>1</sup>, M. Itoh<sup>1</sup>, H. Maki<sup>2</sup>, H. Ninomiya<sup>2</sup>, K. Ohno<sup>2</sup>, M. Oshimura<sup>3</sup>, S. Takashima<sup>4</sup>, Y. Goto<sup>1</sup>.* 1) Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan; 2) Department of Neurobiology, Tottori University School of Life Sciences, Yonago, Tottori, Japan; 3) Department of Molecular and Cell Genetics, Tottori University School of Life Sciences, Yonago, Tottori, Japan; 4) National Institute of Neuroscience, NCNP, Kodaira, Tokyo, Japan.

The gene of amyloid precursor protein (APP) is located on human chromosome 21 (hu-chr21), and accumulation in the brain of amyloid b protein (Ab), as a breakdown product of APP, is thought to be a leading factor for early onset of dementia in Down syndrome (DS) adults because of trisomy for hu-chr 21. Recent studies indicate the hypothesis that Ab can be neurotoxic in vitro and in vivo. Our recent study revealed that up-regulation of transcription factor, E2F-1 was found in elderly DS brains exhibiting the neuropathological features of Alzheimer-type dementia (DAT). Enhanced immunoreactivity for E2F-1 was detected in the pyramidal neurons of the cerebral cortex from DS brains with DAT, in accordance with Ab deposition in the neurons. As to the Ab neurotoxicity in vitro, E2F-1 mediates death of Ab treated cortical neurons. As p53 and p73 are known to be the transcriptional targets of E2F-1, we investigated p53 and p73 expression in DS brains. P73 alone was up-regulated in the pyramidal neurons of DS brains with DAT, implying that the activation of E2F-1-p73 pathway is a critical for Ab-triggered neuronal death in DS brains with DAT. To elucidate further, we generated the model neurons for DS: PC12 cells into which a segment of hu-chr 21 was introduced. The model neurons containing a segment with genes of Down syndrome critical regions exhibited E2F-1-mediated neuronal death with apoptosis. By using the neurons, our major goal is to determine gene dosage effect of what gene(s) of hu-chr 21 causes the overproduction of Ab and neurodegeneration in DS brains.

**Genetic analysis of spinocerebellar ataxia type 6 in a very large kindred from a mountainous isolate of**

**Azerbaijan.** *S.N. Illarioshkin<sup>1</sup>, S.A. Klyushnikov<sup>1</sup>, L.V. Novikova<sup>1</sup>, M.G. Adjamatov<sup>1</sup>, P.A. Slominsky<sup>2</sup>, E.D. Markova<sup>1</sup>, S.A. Limborska<sup>2</sup>, I.A. Ivanova-Smolenskaya<sup>1</sup>.* 1) Department of Neurogenetics, Institute of Neurology, Moscow, Russia; 2) Department of Human Molecular Genetics, Institute of Molecular Genetics, Moscow, Russia.

Autosomal dominant ataxias represent a heterogeneous group of disorders associated with 14 known chromosomal loci. One of the most common forms of dominant ataxias, spinocerebellar ataxia type 6 (SCA6), is caused by moderate CAG repeat expansion in the coding region of the *CACNLIA4* gene on chromosome 19p13.1. We examined a very large consanguineous 5-generation family residing in a mountainous isolate of Azerbaijan in which 42 family members suffered from an autosomal dominant ataxic disorder. Direct DNA analysis identified this disorder as SCA6—the first case of SCA6 in the countries of the former Soviet Union. In total, 27 relatives were examined personally and underwent molecular testing (including 20 affected individuals). Clinical findings included cerebellar ataxia, dysarthria, and mild pyramidal spasticity. On MRI (performed in three patients), the signs of isolated cerebellar atrophy were found; brainstem auditory evoked potentials were relatively spared. The ages at disease onset ranged from 26 to 50 years. There was clear evidence for anticipation in this family, with difference in onset ages between generations of 7-10 years. DNA analyses showed that each of the affected persons inherited one mutant *CACNLIA4* allele with 23 copies of CAG repeats. Therefore, this allele was characterized by genetic stability and the lack of change in CAG repeat length upon transmission in a number of meioses.

*Conclusions.* The present family demonstrated that anticipation in SCA6 may not be related to genetic instability of the expanded CAG repeat-containing allele, and confirmed the complexity of molecular mechanisms underlying the phenomenon of anticipation. Taking into consideration the consanguineous nature of the family under study, we suggest that homozygosity at some hypothetical modifying loci may account for some genetic characteristics of the disorder observed in this SCA6 family.

**Analysis of candidate genes for autism on chromosomes 7q, 2q and 16p.** *E. Maestrini, on behalf of IMGSAC.*

<http://www.well.ox.ac.uk/~maestrin/iat.html>.

Autism is a severe neurodevelopmental disorder, likely to arise on the basis of a complex genetic predisposition. The IMGSAC has completed a whole genome screen for autism susceptibility loci on 86 sib-pairs; regions on 12 chromosomes were further investigated in 66 additional sib-pairs. In the total data set of 152 sib-pairs, chromosome 2q gave the highest result with a MLS of 3.74 at D2S2188, followed by chromosome 7q with a MLS of 3.37 at D7S477, and chromosome 16p with a MLS of 2.93 at D16S3102. Candidate gene studies are in progress in order to isolate the susceptibility genes at these three loci. All coding and functionally relevant regions of candidate genes are being systematically screened for sequence variants using DHPLC and sequencing. Screening is being carried out in a sample of 40-50 autistic subjects chosen from IMGSAC families which are contributing to the linkage peaks on 2q, 7q, or 16p, respectively. This strategy selects for cases who are more likely to carry etiological variants at these loci compared to a random patient sample. All DNA variants with putative functional significance identified in each gene are tested in a control sample to investigate their frequency in the normal population. In addition, intragenic SNPs detected through the screening are tested in the complete IMGSAC family data set for association with autism, using the Transmission Disequilibrium Test. At present, analysis has been completed for four adjacent genes localised to a region in 7q32, which contains an imprinted domain: PEG1/MEST, COPG2, CPA1, and CPA5. No evidence for an etiological variant was found at any of these genes. Given previous reports suggesting a parent of origin-specific effect at the 7q locus, we also tested the hypothesis that imprinting mutations in the PEG1/COPG2 domain might play a role in autism. Both DNA methylation studies and replication timing analysis indicated a normal imprinting regulation of the PEG1/COPG2 domain. Our analysis strongly suggests that it is unlikely that these four genes play a major role in autism aetiology. Other genes currently under investigation include: RLN, DLX5/DLX6, WNT2 on chromosome 7q; DLX1 and DLX2, TBR1, CHN1, GAD1 on chromosome 2q; GRIN2A, CBP and TBX6 on chromosome 16p.

**Genetic association between Alzheimer disease and the polymorphic markers on chromosome 10.** *T. Miki<sup>1</sup>, H. Yamagata<sup>1,2</sup>, M. Matsubara-Tsutsui<sup>1</sup>, J. Nakura<sup>1</sup>, T. Nomura<sup>1</sup>, K. Kohara<sup>1</sup>, K. Kamino<sup>3</sup>, I. Kondo<sup>2</sup>.* 1) Dept Geriatric Medicine, Ehime Univ Sch Medicine, Ehime, Japan; 2) Dept Hygiene, Ehime Univ Sch Medicine, Ehime, Japan; 3) Dept Clinical Neuroscience, Osaka Univ Grad Sch Med, Osaka, Japan.

The apolipoprotein E (APOE) gene is the only known genetic risk factor for late onset Alzheimer disease (LOAD). However, 50% of LOAD cases carry no APOE4 alleles, suggesting that there must be additional risk factors. Recently several reports suggest that there may contain one or more genes on chromosome 10 associated with LOAD by genetic linkage studies. To investigate the association of chromosome 10 markers with the risk of AD, we carried out a case-control study using microsatellite markers D10S1423 (10p) and D10S583(10q). We examined DNA samples of 50 sporadic, early onset AD patients, 146 LOAD patients, and 204 controls by PCR based assays. In D10S1423, ten alleles ranging in size from 206 to 242 bp ((GATA)n) were identified. There was no statistically significant difference in genotype or allele frequency distributions between cases and controls for the polymorphisms at D10S1423. In D10S583, fifteen alleles ranging in size from 187 to 221 bp ((CA)n) were identified. The multiallelic test on all the 15 alleles showed a statistically significant difference in allele frequencies between the LOAD patients and control subjects ( $P = 0.018$ ;  $df=14$ ). Especially, the frequency of the 203bp allele was significantly lower in LOAD patients than controls ( $p<0.007$ ). Carriers of the 203bp allele had a 0.23-fold decreased risk of developing LOAD than noncarriers. The odds ratio for the ApoE4 and the noncarriers of the 203bp allele was 3.68. The results showed that the D10S583 marker is associated with sporadic LOAD, independent of ApoE4 status. Taken together, the present study supports the previous reports that the D10S583 marker is in linkage disequilibrium with the putative LOAD locus (AD6) on chromosome 10q. The marker is located in intron of the kinesin-like 1 gene (KNSL1). The order of the nearby genes is cen-IDE-KNSL1-HHEX-qter. Therefore, these genes are the strong candidates for LOAD.

**Exon 11 of the chromosome 22 gene WKL1, previously shown to exhibit a base pair mutation in rare cases of schizophrenia, was found to contain an insertion/deletion polymorphism, affecting the receptor channel amino acid sequence.** *A. McQuillin<sup>1</sup>, G. Kalsi<sup>1</sup>, H. Moorey<sup>1</sup>, G. Lamb<sup>1</sup>, S. Mayet<sup>1</sup>, D. Quested<sup>2</sup>, H. Gurling<sup>1</sup>.* 1) Molecular Psychiatry lab, University College London, Windeyer Inst. 46 Cleveland St. London, England W1T 4JF UK; 2) Ealing Hospital, North West London Mental Health Trust.

A missense mutation in exon 11 of a novel gene encoding a potassium channel on chromosome 22 was found to be associated with six cases of catatonic schizophrenia in a single large pedigree (Meyer et al, 2001, *Molecular Psychiatry*, 6, 302-306). We have screened exon 11 of the WKL1 gene in 200 cases of schizophrenia by direct sequencing, including cases of catatonic schizophrenia, but could not detect the previously reported mis-sense mutation. We did, however, find an insertion/deletion affecting both the 3 prime end of exon 11 and the neighbouring intron. Preliminary analyses showed that the insertion/deletion was found to be in Hardy Weinberg equilibrium in both cases and controls and that it was probably not associated with schizophrenia. The insertion/deletion is composed of repeated sequence from exon 11 and intron 11 and is predicted to affect the protein structure of WKL1. Our finding suggests that variation in the WKL1 gene can exist without being associated with schizophrenia.

**Updated analysis of MTHFR in American Caucasian lumbosacral myelomeningocele (NTD) families.** *E.C. Melvin<sup>1</sup>, D.G. Siegel<sup>1</sup>, S. Slifer<sup>1</sup>, L. Mehlretter<sup>1</sup>, S. Samal<sup>1</sup>, D.S. Enterline<sup>1</sup>, T.M. George<sup>1</sup>, J.S. Nye<sup>2</sup>, M.C. Speer<sup>1</sup> and NTD Collaborative Group.* 1) Center for Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) Northwestern University Medical Center, Chicago, IL.

Neural tube defects (NTD) are common birth defects with both genetic and environmental causes. Maternal periconceptional folic acid supplementation reduces the recurrence risk for NTDs by up to 70%;, suggesting that genes involved in folate metabolism are feasible candidates. The thermolabile variant of the methylenetetrahydrofolate reductase (MTHFR) gene has been associated with NTDs in some reports, and not associated in other reports. And, reports of heterozygote advantage in NTD cases have been suggested. We updated our original analysis of MTHFR in 65 families (Speer et al, 1997) by evaluating the 677C->T polymorphism in 145 American Caucasian sporadic NTD families in which the proband is affected with lumbosacral myelomeningocele. We found no evidence for 1). allele frequency differences between parents and controls; 2). allele frequency differences between cases and controls; 3). linkage disequilibrium in family triads; 4). maternal-fetal interaction; or 5). heterozygote advantage at MTHFR in these pedigrees (all p values > 0.05). When the 1298A->G polymorphism was considered independently and in tandem with the 677C->T polymorphism, the results are consistent, showing no evidence for involvement of MTHFR in these families. These data confirm our previous report of no association of lumbosacral myelomeningocele with MTHFR, suggesting that this gene does not play a major role in the development of NTDs in these families. Reported associations may be population-specific or mediated by environmental factors not relevant or common in our sample. Alternatively, associations with MTHFR may be dependent on level of lesion.



**Polymorphism Analysis of the human striatum-enriched CalDAG-GEFI gene with Japanese schizophrenia patients.** *H. Kawasaki<sup>1</sup>, H. Mitsuyasu<sup>1</sup>, N. Maeda<sup>1</sup>, T. Tahira<sup>2</sup>, H. Ninomiya<sup>3</sup>, Y. Fukumaki<sup>4</sup>, D.E. Housman<sup>5</sup>, A.M. Graybiel<sup>6</sup>, K. Hayashi<sup>2</sup>, N. Tashiro<sup>1</sup>.* 1) Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan; 2) Div Genome Analysis, Res Ctr Genet Info, Med Inst Bioregulation, Kyushu Univ, Fukuoka, Japan; 3) Dazaifu Pref Hosp, Fukuoka, Japan; 4) Div Disease Genes, Res Ctr Genet Info, Med Inst Bioregulation, Kyushu Univ, Fukuoka, Japan; 5) Cent for Cancer Res, MIT, Cambridge, MA; 6) Dept Brain and Cognitive Sci, MIT, Cambridge, MA.

We previously reported the isolation of striatum-enriched novel guanine nucleotide exchange factor (GEF) that has both calcium (EF-hand) and diacylglycerol (DAG) binding domains (CalDAG-GEFI) and the presence of its ortholog, CalDAG-GEFII (identical to RasGRP, Ebinu et al., 1998). These genes are part of the novel second-messenger regulated GEF gene family whose GEF activities are regulated by the binding of second-messenger molecules such as cAMP, calcium and DAG. It is shown that CalDAG-GEFI proteins are localized at the synaptic terminals of GABAergic output neurons in the striatum (Kawasaki et al., 1998). Since the dysregulation of the striatal GABAergic output neurons are implicated in the pathophysiological mechanisms of schizophrenia, CalDAG-GEFI gene can be a good candidate for molecular studies of schizophrenia. All 17 exons of human CalDAG-GEFI were amplified by PCR to isolate single nucleotide polymorphisms (SNPs) using 12 Japanese individuals. Initial screening was by dHPLC, subsequently, the SNPs were evaluated by direct sequencing. As a result, total 5 SNPs including one previously reported were isolated. We have genotyped total 4 SNPs (2 newly isolated (IVS9(-92)A/Del and IVS10(+12)C/T), and 2 registered (IVS9(-44)C/T (rs556356) and IVS10(-43)C/T (rs553618)) in the schizophrenia patients (n=51) and normal controls (n=52) (Japanese population) to evaluate the association between schizophrenia phenotype and these markers. Direct sequencing and single nucleotide extension were employed as genotyping methods. No significant differences in the allele frequencies of the novel SNPs were found. Two other registered SNPs were monomorphic in the Japanese population.

**Polymorphism analysis of the human dopamine receptor (DRD4) gene with Japanese schizophrenia patients.** *H. Mitsuyasu<sup>1</sup>, H. Kawasaki<sup>1</sup>, N. Maeda<sup>1</sup>, A. Tahara<sup>1</sup>, Y. Matsuo<sup>1</sup>, T. Tahira<sup>2</sup>, H. Ninomiya<sup>3</sup>, Y. Fukumaki<sup>4</sup>, K. Hayashi<sup>2</sup>, N. Tashiro<sup>1</sup>. 1) Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan; 2) Div Genome Analysis, Res Ctr Genet Info, Med Inst Bioregulation, Kyushu Univ, Fukuoka, Japan; 3) Dazaifu Pref Hosp, Fukuoka, Japan; 4) Div Disease Genes, Res Ctr Genet Info, Med Inst Bioregulation, Kyushu Univ, Fukuoka, Japan.*

The human dopamine D4 receptor (DRD4) gene is considered to be a good candidate for susceptible genetic components of schizophrenia, because an atypical antipsychotic drug, clozapine has a relatively high affinity to DRD4 (Van Tol et al., 1991), and elevation of the density of DRD4 and DRD4 mRNA expression were found in the postmortem brains of schizophrenia patients (Seeman et al., 1993, Stefanis et al., 1998). Many polymorphisms have been reported in the DRD4 gene locus. Previously we reported 9 novel single nucleotide polymorphisms (SNPs) in the upstream region of the DRD4 gene in the Japanese population (Mitsuyasu et al., 1999). We genotyped 5 SNPs in the region with 208 Japanese schizophrenia patients and 210 normal controls. (Mitsuyasu et al., 2001). We collected total 32 polymorphisms from a 4.9-kb region of entire DRD4 gene, which includes 27 SNPs and 5 other polymorphisms (repeat; 3, deletion; 2), based on our experiments, database, and published sources. Most of them were accumulated within approximately 1-kb area where exon 1 and the promoter region are located. Out of them, we genotyped 8 polymorphisms (tandem duplication; 1, known SNPs; 4, novel SNPs; 3) in the upstream region of the DRD4 gene with the Japanese schizophrenia patients (n=69-110) and normal controls (n=63-95) by PCR and direct sequencing method. We analyzed the association between schizophrenia patients and these polymorphisms by chi square test. Allele and genotype frequencies between the Japanese schizophrenia patients and normal controls were not significantly different. Genotyping of other polymorphisms of DRD4 gene is in progress.

**Linkage and association of the glutamate receptor 6 gene with autism.** *S. Jamain*<sup>1</sup>, *C. Betancur*<sup>2</sup>, *H. Quach*<sup>1</sup>, *A. Philippe*<sup>2</sup>, *M. Fellous*<sup>1</sup>, *B. Giros*<sup>2</sup>, *C. Gillberg*<sup>3</sup>, *M. Leboyer*<sup>2</sup>, *T. Bourgeron*<sup>1</sup>. 1) Immunology, Pasteur Institute, Paris, France; 2) INSERM U513, Faculte de Medecine de Creteil, 94000 Creteil, France; 3) Department of Child and Adolescent Psychiatry, Goteborg University, 41119 Goteborg, Sweden.

A genome scan was previously performed and pointed chromosome 6q21 as a candidate region for autism. This region contains the glutamate receptor 6 (GluR6 or GRIK2) gene, a functional candidate for the syndrome. Glutamate is the principal excitatory neurotransmitter in the brain and is directly involved in cognitive functions such as memory and learning. We used two different approaches, the affected sib-pair (ASP) method and the transmission disequilibrium test (TDT), to investigate the linkage and association between GluR6 and autism. The ASP method, conducted on 59 families, showed a significant excess of allele sharing, generating an elevated multipoint maximum LOD score (GENEHUNTER-PLUS NPL= 3.28; P = 0.0005). Using 107 additional families with a single affected child, a significant maternal transmission disequilibrium was observed (TDT linkage P = 0.0004). Furthermore, TDT (with only one affected proband per family) and Haplotype Relative Risk (HRR) analyses showed significant association between GluR6 and autism (TDT association P = 0.008; HRR P = 0.01). In contrast to maternal transmission, paternal transmission of GluR6 alleles was as expected in the absence of linkage, suggesting a maternal effect such as imprinting. Mutation screening was performed in 33 affected individuals, revealing several nucleotide polymorphisms (SNPs), including one amino acid change (M867I) found in 8% of the autistic subjects. This change takes place in a highly conserved domain of the protein and seems to be more maternally transmitted than expected to autistic males (P = 0.007). Taken together, these data suggest that GluR6 is in linkage disequilibrium with autism.

**QTL Association Analysis of the Dopamine D4 and Dopamine Transporter Genes in Adult ADHD. P.**

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Converging lines of evidence support a key role for the dopamine system in Attention Deficit Hyperactivity Disorder (ADHD). The genes for the dopamine D4 receptor and the dopamine transporter (DRD4 and SLC6A3) have been studied in a number of child ADHD samples. The 7-repeat alleles of the dopamine D4 receptor gene have been reported to increase the risk for ADHD in children while the investigation on the SLC6A3 3'UTR VNTR has shown less clear results. We investigated whether DRD4 and SLC6A3 alleles increase the risk for the variant of ADHD that persists in adulthood. We tested for the presence of association considering the Adult ADHD phenotype as a categorical and as a continuous trait as evaluated with the Brown Adult ADD scale. 153 Adult ADHD patients were studied (mean age: 37.69; S.D.+11.86; range: 19 to 69; 101/52 male female). We found evidence of association of the DRD4 7 alleles with Adult ADHD considered as a categorical phenotype (Chi-squared for genotype-wise TDT = 11.587814, 5 df, p = 0.041). No association was detected between either the DRD4 or the SLC6A3 when we performed a linear regression considering the risk alleles as the independent variable and the Total Brown scores, that measure ADHD core symptomatology as the independent variable. However the patients with the 7 repeat alleles have higher but not significant Brown total scores (means 86.02+ 22.10) when compared to patients without the 7 allele (mean = 82.42; t = 0.993; p = 0.32). Our results confirm that, as in child samples from other studies, the DRD4 7 is conferring a small risk in the adult variant of ADHD.

**Analysis of mutations in microtubule-associated protein tau (MAPT) gene in fronto-temporal dementia (FTD) in Japanese population.** *N. Maeda<sup>1</sup>, H. Kawasaki<sup>1</sup>, H. Mitsuyasu<sup>1</sup>, K. Ogomori<sup>1</sup>, M. Takita<sup>2</sup>, A. Tahara<sup>1</sup>, Y. Matsuo<sup>1</sup>, T. Tahira<sup>3</sup>, K. Hayashi<sup>3</sup>, N. Tashiro<sup>1</sup>.* 1) Dept Neuropsychiatry, Kyushu Univ, Fukuoka, Japan; 2) Dept Psychiatry, Imazu Red Cross Hosp, Fukuoka, Japan; 3) Div Genome Analysis, Res Ctr Genet Info, Med Inst Bioregulation, Kyushu Univ, Fukuoka, Japan.

Fronto-temporal dementia (FTD) is the second largest degenerative dementia group after Alzheimer's disease (AD). The symptoms of FTD are characterized by progressive personality and behavioral changes. However, memory and visual spatial orientation are preserved in relatively late stage in contrast to AD. It is shown that a positive familial aggregation can be observed in almost half of the FTD cases. It is also reported that approximately 10-40% of FTD cases with a positive family history are caused by mutations in the microtubule-associated protein tau (MAPT) gene. Exon and intronic mutations of MAPT gene result in several neurodegenerative disorders such as AD, Pick's disease, FTD, corticobasal degeneration and progressive supranuclear palsy. The different tau mutations may result in disturbances in the interactions of the protein tau with microtubules, resulting in hyperphosphorylation of tau protein, assembly into filaments, and subsequent cell death. So far, total 13 mutations which are responsible for FTD have been reported. In this study, we have collected DNA samples from 26 FTD in Japanese population. We have analyzed 13 reported mutations (1 mutation in exon9 (Gly272Val), 4 in exon 10 (Asn279Lys, D280, Pro301Leu and Ser305Asn), 1 in exon 12 (Val337Met), 2 in exon 13/14 (Gly389Arg and Arg406Trp) and 5 stem-loop mutations in intron 10 (+1G/A, +3G/A, +13A/G, +14C/T, +16C/T) of human MAPT gene in Japanese FTD patients (n = 7-14). Direct sequencing method was employed for this analysis. We found no reported mutations in the Japanese FTD samples. These results suggest that MAPT gene may not be involved in the pathophysiological mechanisms of FTD in Japanese population. Further investigation of mutations in the other exons and introns of MAPT gene is in progress.

**EXPRESSION OF DISC-1 AND ITS POSSIBLE FUNCTIONS: IMPLICATION FOR THE PATHOGENESIS OF SCHIZOPHRENIA.** *Y. Ozeki<sup>1,2</sup>, K. Fujii<sup>1</sup>, A. Kamiya<sup>1</sup>, H. Otsuki<sup>1</sup>, X. Luo<sup>2</sup>, N. Yamada<sup>1</sup>, R.L. Margolis<sup>2</sup>, M. Okawa<sup>1</sup>, S.H. Snyder<sup>2</sup>, C.A. Ross<sup>2</sup>, A. Sawa<sup>2</sup>.* 1) Psychiatry, Shiga Univ. of Med. Sci, Otsu, Shiga, Japan; 2) Dept. of Neuroscience and Psychiatry, Johns Hopkins Univ. Sch. of Med.

DISC-1 (Disrupted-In-Schizophrenia) is a transcript whose open reading frame is disrupted by a balanced chromosomal translocation in a large Scottish family. The chromosomal translocation is highly associated with major mental illnesses in the family, suggesting that DISC-1 may confer vulnerability to major mental illnesses, especially schizophrenia. In our preliminary in situ hybridization experiments in rat, we found high expression of DISC-1 messenger RNA in olfactory bulb, moderate expression of DISC-1 in hippocampus and cortex, and little in cerebellum. This could be consistent with a function in olfaction, limbic functioning, cognition, and memory, processes thought to be involved in schizophrenia. To evaluate protein expression of DISC-1, we have developed an antibody against DISC-1. Using the antibody, we have confirmed the existence of DISC-1 at protein level, which is enriched in olfactory bulb and hippocampus. To clarify its biological significance, we have initiated yeast-2-hybrid screening. Identification of DISC-1 interactors will help elucidate possible pathways in which DISC is involved, and hence may provide insight into the normal function of DISC-1. We have obtained several possible interactors of DISC-1, most of which belong to actin-associated cytoskeletal components. We hypothesize that DISC-1 and its interactors form a functional multiprotein complex beneath the plasma membrane, perhaps in conjunction with ion channels and receptors.

**WNT2 and Autism.** *T.H. Wassink<sup>1</sup>, J. Piven<sup>2</sup>, V.J. Vieland<sup>1,3</sup>, J. Juang<sup>4</sup>, R.E. Swiderski<sup>5</sup>, J. Pietila<sup>5</sup>, C.C. Searby<sup>5</sup>, T. Braun<sup>5,6</sup>, G. Beck<sup>5</sup>, S.E. Folstein<sup>7</sup>, J.L. Haines<sup>8</sup>, V.C. Sheffield<sup>5</sup>.* 1) Dept Psychiatry, University of Iowa College of Medicine; 2) Neurodevelopmental Disorders Research Center and Department of Psychiatry, University of North Carolina; 3) Department of Biostatistics, University of Iowa College of Public Health; 4) Department of Statistics and Actuarial Science, University of Iowa; 5) Department of Pediatrics and the Howard Hughes Medical Institute, University of Iowa College of Medicine; 6) Interdepartmental Genetics Ph.D. Program, University of Iowa; 7) Department of Psychiatry, Tufts University College of Medicine; 8) Program in Human Genetics, Vanderbilt Medical Center.

The WNT family of genes influence development, including development of the central nervous system. WNT2 is located in the 7q31-33 autism linkage region and is adjacent to an autism chromosomal breakpoint. A mouse knockout of Dvl1, (Dvl genes are essential for WNT functioning) exhibits a behavioral phenotype characterized primarily by diminished social interaction. We, therefore, screened WNT2 as an autism candidate gene. We identified two families containing non-conservative coding sequence variants that segregated with autism in those families. We also identified LD between a WNT2 3'UTR SNP and our sample of autism families. The LD arose almost exclusively from a subgroup of affected sibling pair families defined by the presence of severe language abnormalities, and was also found to be associated with the evidence for linkage to 7q from our previously published genome-wide linkage screen. Furthermore, expression analysis demonstrated WNT2 expression in the human thalamus. To follow up these preliminary findings, we have assessed the functional relevance of the WNT2 sequence variants in cell-based transfection assays. We have identified and genotyped more SNPs in and around WNT2 in order to further characterize the LD. We have also screened a number of genes related to WNT function, such as Frizzled-1 and Frizzled-9, as autism candidate genes. No coding sequence changes unique to autism have yet been found in these genes, though a number of polymorphisms useful for testing for LD have been identified.

**Distribution of Parkin gene mutations in subgroup of Parkinson's disease.** *A. Parsian*<sup>1</sup>, *B. Racette*<sup>2</sup>, *R. Sinha*<sup>1</sup>, *J. Perlmutter*<sup>2</sup>. 1) Birth Defects Center, Univ Louisville Hlth Sci Ctr, Louisville, KY; 2) Department of Neurology, Washington Univ, St. Louis, MO.

Mutations in parkin gene are major cause of autosomal recessive juvenile parkinsonism (AR-JP) but their role in idiopathic Parkinson's disease (PD) is not clear. There are only few reports that suggest a possible association between parkin mutations and early onset atypical PD, but there is no conclusive evidence in support of these findings. In order to evaluate the pathogenesis of parkin gene in subgroups of PD, we screened a sample of PD patients (N= 433) and normal controls (N= 110) with four most common mutations in different exons of the parkin gene. A total of 10 mutations in exon 4 and 2 in exon 7 in form of heterozygote were detected in PD group and none in controls. However, no mutation was observed in exons 6 and 8 in any group. There was no correlation between any mutation and family history or age of onset. We concluded that these mutations are not playing any critical role in the development of PD among our patients.



**The human SCA3 gene: Genomic organization and functional promoter analysis.** *I. Schmitt, B. Evert, H. Khazneh, T. Klockgether, U. Wuellner.* Neurology, University of Bonn, Bonn, Germany.

Spinocerebellar ataxia type 3 (SCA3) is a progressive neurodegenerative disorder caused by pathological expansion of a CAG motif within the translated region of the *SCA3* gene. Although a partial cDNA sequence has been known for about seven years molecular features of the genomic structure of the gene and the transcription start side are still unknown. Furthermore Northern blot studies indicate that 60% of the *SCA3* cDNA sequence (5' and 3' UTR) are missing.

We have analysed the genomic structure and identified the putative promoter region of the *SCA3* gene. 5'-RACE experiments and the comparison with the rat *SCA3* cDNA Sequence extended the known 5'-end of the human cDNA by 44 bp. 3'-RACE analysis lead to a complete *SCA3* cDNA sequence of about 6 kb. The gene consists of at least 11 exons and spans a region of about 47 kb. Analysis of a 1 kb DNA fragment at the 5'-flanking region of this gene revealed the presence of a TATA-less promoter containing GC-rich regions, a CCAAT and multiple potential Sp1 binding sites. Luciferase reporter assays performed in human and non-human cell lines demonstrated a promoter activity of this fragment in the lower range which is in accordance with the low RNA levels detected in previous expression studies. Subsequent 5' deletions of this fragment confined promoter elements to a 600 bp region upstream of the translational start side.

**Further evidence that genes outside of the folate-homocysteine metabolic axis influence neural tube development: the human *T* locus and spina bifida.** A.S. Whitehead, S. Barbaux, M. McDonnell, K. Hoess, L.E. Mitchell. University of Pennsylvania School of Medicine, Philadelphia, PA.

Studies establishing that prenatal folic acid supplementation can prevent up to 70% of all neural tube defects (NTD) have spurred interest in the relationship between genes involved in folate-homocysteine metabolism and NTD risk. However, not all NTD are prevented by folic acid, and animal data suggest that neural tube development is influenced by a variety of genes. The human *T* locus is an attractive candidate gene for NTD, since mutations in the mouse homologue (Brachyury) produce phenotypes similar to human spina bifida. Previous studies from Europe have provided evidence that the TIVS7 polymorphism of human *T* is associated with NTD risk. Specifically, the TIVS7-2 allele was found to be preferentially transmitted from heterozygous parents to their affected offspring. This finding has thus far not been confirmed in the U.S., but the single published study is based on a small sample. As the relative contribution of any gene to the overall risk of spina bifida is likely to vary across populations, it is essential that the role of this variant be determined in a contemporary U.S. sample. Consequently, the transmission disequilibrium test was used to evaluate the TIVS7 polymorphism in data from spina bifida family trios ascertained through two institutions in the Delaware Valley. Consistent with the European studies, the TIVS7-2 allele was transmitted in excess from heterozygous parents to affected offspring (28/46). In addition, these heterozygous parents transmitted the TIVS7-2 allele less frequently than expected to their unaffected offspring (12/32). The difference in the pattern of transmissions to affected and unaffected offspring was significant ( $P=0.04$ ). Relative to TIVS7-1 homozygotes, the risks to heterozygotes and TIVS7-2 homozygotes, were estimated to be increased by 1.4 and 2.5-fold, respectively. Hence, these data provide further evidence that the TIVS7 variant, or a variant with which it is in linkage disequilibrium, is a risk factor for spina bifida and help to refine our understanding of the "genetic architecture" of this condition.

**Association of a variation in the brain-derived neurotrophic factor (BDNF) gene with familial Parkinson's disease.** *R. Sinha*<sup>1</sup>, *B. Racette*<sup>2</sup>, *J.S. Perlmutter*<sup>2</sup>, *A. Parsian*<sup>1</sup>. 1) Birth Defects Center, Univ Louisville Hlth Sci Ctr, Louisville, KY; 2) Department of Neurology, Washington Univ, St. Louis, MO.

Idiopathic Parkinson's disease (PD) is one of the most common neurodegenerative disorders with the frequency of 1% in people over the age of 50 years. Most cases of PD are sporadic and only 20-25% have a positive family history with complex mode of inheritance. Brain-derived neurotrophic factor (BDNF) is one of the growth factors with dopaminergic activity. Intrathecal infusion of BDNF reduces loss of dopamine neurons and ameliorates parkinsonism in the MPTP treated monkey model of PD. In addition, it has been shown that BDNF has therapeutic effects on PD. To determine the role of BDNF gene in the development of familial and sporadic PD, we screened our sample with a variation (C270T) in the 5' non-coding region of the gene. The sample comprised of 170 patients with and 227 without a positive family history for PD and 196 matched controls. Comparison of the allele frequency between familial PD and normal controls was highly significant ( $p= 0.0002$ ) but not between sporadic PD and controls. Both PD groups were categorized based on age of onset (AON) of <30, <50, and >50 years. There was no significant difference between the three AON subtypes of PD in familial and sporadic groups. However, we identified two patients in AON of <30 and one in <50 years group who were homozygous for mutated allele. Our data indicate the possibility of linkage disequilibrium between this variation and a mutation in coding region of BDNF gene. Our results also indicate that BDNF plays a role in the development of familial PD that is consistent with the results of clinical and animal model studies.  $\leq$ .

**Neurological dysfunction in a mouse model of SCA7 is associated with proteolytic cleavage of polyglutamine-expanded ataxin-7.** *B.L. Sopher<sup>1</sup>, Y.H. Fu<sup>2</sup>, R.T. Libby<sup>1</sup>, Y. Kinoshita<sup>3</sup>, D.D. Einum<sup>4</sup>, J. Huang<sup>5</sup>, D.E. Possin<sup>5</sup>, R.S.*

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Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant disorder characterized by gait ataxia and retinal degeneration, and is caused by a CAG repeat expansion. To determine the mechanism of ataxin-7 polyglutamine neurotoxicity, we produced transgenic mice that express ataxin-7 with 24 or 92 glutamines using the murine prion protein promoter. After generating antibodies against human ataxin-7, we probed Western blots from the 92Q mice and identified an 130 kD band, confirming expression of full-length ataxin-7. IHC analysis indicates that ataxin-7 protein in the 92Q mice forms nuclear aggregates in neurons of the cortex and brainstem. Furthermore, the ataxin-7 transgene is expressed in the pontine nucleus, inferior olive, and cerebellar granule cell layer, all regions affected in patients. While breeding the 92Q mice, it soon became apparent that they display a neurological phenotype, presenting as an unsteady gait. The gait ataxia progressed until the mice start falling, become less explorative, and develop a chronic tremor. Such affected mice also show decreased survival. Based on RT-PCR analysis, the expression levels of the ataxin-7 92Q transgene are roughly twice that of endogenous murine ataxin-7. PrP-SCA7-c24Q transgenic mice with comparable expression levels, however, are asymptomatic. An intriguing feature of our SCA7 model is the presence of an N-terminal truncation fragment that migrates at ~ 55 kD on SDS-polyacrylamide gels and may be detected in 92Q mice with the 1C2 antibody. As ataxin-7 92Q aggregates do not immunostain with antibodies raised against the C-terminal region, our data suggest that proteolytic cleavage is occurring prior to ataxin-7 aggregation, and thus may be required for the neurotoxicity.

**Association of HOPA polymorphisms with Major Depression and Phobia.** *R.A. Philibert, K. Kaspers, D. Langbehn, E.P. Troughton, R. Yucuis, H.K. Sandhu, R.J. Cadoret.* Psychiatry Research/MEB, Iowa City, IA.

Thyroid hormone has a prominent role in the development and homeostasis of the Central Nervous System. Consequently, genes participating in thyroid hormone receptor mediated signal transduction are prime candidates for neuropsychiatric illness susceptibility factors. .Previously, we have associated exonic polymorphisms in a Xq13 thyroid receptor co-activator named HOPA with a modest increase in vulnerability to a broad spectrum of neuropsychiatric illness, including depression, psychosis and hypothyroidism. To test and extend these findings, we have now examined the relationship between HOPA polymorphisms and neuropsychiatric illness in a cohort of Iowa adoptees. Consistent with our prior findings, HOPA polymorphisms were associated with an increased risk for major depression as well as phobic conditions. There was suggestive evidence that the increased psychiatric morbidity in these subjects could represent epistasis: e.g., an interaction between the HOPA variant and a genetic diathesis for another psychiatric condition such as biologic parent antisocial behavior. Since thyroid function tests were not available on these subjects, used obesity as a surrogate for hypothyroidism. Interesting, HOPA polymorphism were associated with a marked increased risk for obesity. We conclude that HOPA polymorphisms may be a moderate risk factor for increased susceptibility to a broad spectrum of neuropsychiatric illness and hypothesize that the type of illness manifested might be related to a separate genetic diathesis. Further studies are needed to better define the syndrome associated with HOPA polymorphisms and the molecular mechanisms through which they occur. To this end, a larger adoptee sample would allow an answer to the question of interactions between genetic diatheses for psychopathology and HOPA variant, and also allow testing of gene-environment interactions to control for psychiatric outcomes which better reflect the genotype.

**Candidate gene screening on Japanese autism patients.** *T. Yamagata*<sup>1</sup>, *H. Li*<sup>1</sup>, *M. Mori*<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, Minamikawachi, Tochigi, Japan; 2) Department of Pediatrics, Kouri Hospital, Kansai Medical University, Neyagawa, Osaka, Japan.

Several candidate genes were analyzed for possible causative mutations among autistic children in Japanese patients using their DNA from lymphoblastoid cells. Patients diagnosed as pervasive developmental disorder or autism according to the criteria of DSM-IV were enrolled in this study after the informed consents by their parents. Up to one hundred autistic patients were enrolled. The genes studied included those of peptide hormones and their G-protein-coupled receptors, such as secretin and gastrin releasing peptide receptor (GRPR), and methylation-related genes, such as MeCP2, MBD1, MBD2 and MBD3. All exons and promoter regions of these genes were amplified by PCR and the mutation was screened by DHPLC, and finally confirmed by direct sequencing. We previously reported the base changes in E-box and GC-box, and two missense mutations in secretin gene screening on autism patients. This time we increased the patients including six patients who responded to secretin treatment. No causative mutation was detected in secretin gene on newly involved patients even in the patients who responded to the treatment with secretin. And also no causative mutation was detected in GRPR. We found two missense mutations and some base changes in introns near the exons in MECP2, but they were considered as polymorphism. There were some base changes in MBD1, MBD2 and MBD3 but we could not confirm their contribution to the disease. The fact that no causative mutations were detected on secretin gene in the patients who responded to secretin treatment suggested the involvement of the secretin receptor or the processes of intracellular signal transduction after secretin binding can be responsible for the development of their autism. The methylation-related genes were highly polymorphic. We need to analyze these genes further to clarify the contribution to autism.

**Identification of a functional variant in the NMDAR1 receptor subunit resulting in altered effects of ethanol.** *J.G.*

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The N-methyl-D-aspartate (NMDA) receptors are perhaps the most well understood subtype of excitatory amino acid receptors. These receptors play crucial roles in physiological processes such as synaptogenesis and neuronal plasticity. Dysfunctions of NMDA receptors seem to play a crucial role in the neurobiology of disorders including Parkinson's disease, Alzheimer's disease, epilepsy, and ischemic stroke. NMDA receptor function may also be pivotal to the pathophysiology of addictive behaviors, including alcoholism. We have undertaken a project to discover functional polymorphisms in NMDA receptors. Denaturing high performance liquid chromatography (dHPLC) was used to identify candidate sequence variants in PCR amplified products obtained from a panel of genomic DNAs from 500 unrelated individuals of diverse clinical and ethnic background. DNA sequence variants were confirmed by direct sequencing of PCR products and in some cases, by RFLP analysis. Using this approach, we identified seven gene variants in NR1 and NR2B. One of which resulted in an amino acid change. All of the variants were previously unidentified. The gene variant 8396G>A resulted in a cyctine to tyrosine substitution at position 744. When expressed in human embryonic kidney cells with the native NR2A subunit, this receptor variant demonstrated significant alterations in electrophysiology, in terms of the effects of ethanol upon the receptor. Significant functional changes such as these could result in the predisposition of an individual to alcohol abuse.

**Neurodegeneration and mild gait abnormalities in Huntington's disease knock-in mice.** *V.C. Wheeler<sup>1</sup>, C.-A. Gutekunst<sup>2</sup>, V. Vrbancac<sup>1</sup>, L. Lebel<sup>1</sup>, J.-P. Vonsattel<sup>3</sup>, J.F. Gusella<sup>1</sup>, M.E. MacDonald<sup>1</sup>.* 1) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA; 2) Department of Neurology, Emory University School of Medicine, Atlanta, GA; 3) Division of Neuropathology, College of Physicians and Surgeons of Columbia University, New York, NY.

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disorder. The expansion of a CAG repeat in the HD gene produces an elongated glutamine tract in the huntingtin protein, which, through an unknown mechanism results in the loss of medium spiny neurons in the striatum.

We have generated precise genetic mouse models of HD by inserting expanded CAG repeats into the homologous mouse (*Hdh*) locus. Mice expressing mutant huntingtin containing 92 and 111 glutamine repeats (*HdhQ92* and *HdhQ111*) show a progressive cellular phenotype. This phenotype is glutamine length-dependent, dominantly inherited and selective for medium-spiny striatal neurons, providing strong evidence that the underlying process is important in HD pathogenesis.

We have investigated the role played by wild-type huntingtin by examining the cellular phenotype in hemizygous *HdhQ111/Hdhex4/5* or *HdhQ92/Hdhex4/5* mice expressing one copy of mutant huntingtin but no wild-type huntingtin. No difference was found between hemizygous and heterozygous mice demonstrating that this phenotype is neither slowed nor accelerated by wild-type huntingtin.

At two years of age *HdhQ111* mice show striatal neurodegeneration, increased immunoreactivity to glial fibrillary acidic protein and a subtle gait abnormality. These findings support the idea that the *Hdh* knock-in mice, at younger ages, are equivalent to presymptomatic HD gene carriers, in whom pathogenic processes are underway, but who have yet to display overt clinical symptoms. Therefore, the mice provide a resource to understand the early cellular changes attributable to mutant huntingtin that will eventually lead to cell death, and to test drugs which may act in these early pathways.



**Genetic analysis of Chromosome X in migraine families.** *T. Wieser*<sup>1, 2</sup>, *J. Pascual*<sup>3</sup>, *M. Barmada*<sup>1</sup>, *M. Soso*<sup>1</sup>, *A. Oterino*<sup>3</sup>, *K. Gardner*<sup>1,4</sup>. 1) Neurology, Human Genetics, University of Pittsburgh, Pittsburgh, PA; 2) Neurology, Martin-Luther University of Halle, Germany; 3) Neurology, University Marques de Valdecilla, Spain; 4) Veterans Administration Medical Center, Pittsburgh, PA.

**Objective:** Investigate 66 families with migraine and possible X dominant inheritance for evidence of allele sharing, linkage to Chromosome X. **Background:** Familial clustering of migraine is recognized but the disorder is thought to be genetically complex since it does not consistently follow Mendelian inheritance patterns. We previously recognized a paternal bias for transmission of migraine to daughters by segregation analysis of 60 families. Others have proposed that female predominance for migraine is not entirely attributed to hormonal factors. An X-linked dominant component and locus at Xq24-28 has been suggested for familial migraine. **Methods:** We screened the entire X chromosome in 66 families diagnosed according to IHS criteria. Families with possible male to male transmission were excluded (five). Eighteen markers (ABI PRISM Mapping Set, Panel 28) spanning the entire X chromosome spaced 10cM apart were genotyped using a 3700 ABI PRISM DNA Analyser. Alleles were assigned using the GeneScan Analysis software. Analysis for affected relative allele sharing and linkage was performed using ALLEGRO, VITESSE 1.1 programs and the maximized maximum LOD score (MMLS) method with a variety of allele frequencies, phenocopy rates, penetrances. **Results:** Eighty multi-generational families and 14 sibships were ascertained from Spain (27), Germany (18) and America (35) among 478 total samples. One hundred and fifty-five had migraine without aura, 101 migraine with aura, 32 hemiplegic migraine, and 44 were of uncertain type (IHS 1.7). Linkage analysis of 66 families, all forms of migraine showed combined LOD 1.53, theta 0 at marker DXS8051, Xp22.22 using dominant model, 80% penetrance, 5% phenocopy rate, 20% allele frequency. **Conclusion:** Our results suggest further analysis of this region and Chromosome X is warranted for identification of migraine susceptibility loci. This study was supported by the AHS/Pfizer (KG), AHA(KG), Martin -Luther Univ of Halle, Germany (TW), Foundation Marques de Valdecilla (JP).

**Murine KvDMR1, a putative imprinting control region, exhibits enhancer-blocking activity in a soft agar colony forming assay.** *M.J. Higgins, G.V. Fitzpatrick.* Department of Cancer Genetics, Roswell Park Cancer Inst, Buffalo, NY.

The finding that imprinted genes are often clustered in large imprinted domains implies that the mono-allelic expression of these genes could be regulated by shared cis-acting DNA sequence(s). The best candidates for such regulatory elements are differentially methylated CpG islands characteristic of imprinted genes. The 1 Mb imprinted domain on mouse distal chromosome 7 (human 11p15.5) consists of at least eight imprinted genes. The differentially methylated region (DMR) located between *Igf2* and *H19* has been shown to play a role of an imprinting control region (ICR) as it is important for the proper imprinting of both genes. This ICR functions as a chromatin insulator that, when unmethylated, blocks communication between *Igf2* and its enhancers. Interestingly, the deletion of *Igf2/H19* ICR does not affect imprinting of the genes upstream of *Ins*, suggesting that there must be an additional ICR in the domain. We have shown that targeted deletion of KvDMR1, a differentially methylated CpG island within the *Kcnq1* gene, in mouse resulted in the loss of imprinting of several distal chromosome 7 genes (Fitzpatrick et al, this meeting). To address the possible mechanisms by which KvDMR1 normally suppresses the genes on paternally inherited chromosome, this element was tested for enhancer-blocking activity in a soft agar colony-forming assay using stably integrated constructs. A 3.5 kb DNA sequence containing KvDMR1 was sub-cloned (in different orientations) between the Ed enhancer and the Vd1 promoter fused to the neo gene (both the enhancer and the promoter are from human TCR locus), or upstream of the enhancer. We found that when KvDMR1 was placed between the promoter and the enhancer, the number of neo-resistant colonies was reduced by 95% compared to the control. This effect was dependent on the orientation of KvDMR1 relative to the promoter. Positioning KvDMR1 upstream of the enhancer did not suppress the number of colonies. These results are consistent with KvDMR1 being an insulator rather than a silencer. The possible cause of the orientation-dependent effect of the KvDMR1 insulation is being investigated. Funded by NIH/NCI Grant CA63333.

**Monozygotic twins discordant for Beckwith-Wiedemann syndrome also have different patterns of KvDMR1 methylation and KCNQ10T1 allelic transcription in the chromosome 11p15 imprinted region.** *R. Weksberg<sup>1</sup>, J. Nishikawa<sup>1</sup>, Y. Fei<sup>1</sup>, C. Shuman<sup>1</sup>, T. Stockley<sup>4</sup>, L. Best<sup>5</sup>, D. Chitayat<sup>1</sup>, J. Cameron<sup>1</sup>, M. Li<sup>1</sup>, P. Sadowski<sup>2</sup>, J. Squire<sup>3</sup>.* 1) Hospital for Sick Children & Division of Metabolic & Clinical Genetics and Department of Paediatrics; 2) Molecular & Medical Genetics, University of Toronto; 3) Ontario Cancer Institute & Departments of Laboratory Medicine, Pathobiology, & Medical Biophysics, University of Toronto; 4) Hospital for Sick Children, Departments of Paediatric Laboratory Medicine and Molecular & Medical Genetics, University of Toronto; 5) University of North Dakota.

An intriguing aspect of the Beckwith-Wiedemann syndrome (BWS), a disorder of growth regulation and tumor predisposition, is the apparent excess of female monozygotic (MZ) twins discordant for this syndrome. Less frequently, discordant male MZ twin pairs are reported. BWS is associated with a variety of genetic and epigenetic alterations of imprinted genes on human chromosome 11p15.5. These alterations include paternal uniparental disomy, and imprinting defects at KvDMR1/KCNQ10T1. Mechanisms to explain discordance in BWS MZ twin pairs include postzygotic events leading to skewed X inactivation or epigenetic changes such as, paternal 11p15 UPD in the affected twin only. In our study of KCNQ10T1 transcription and KvDMR1 methylation in monozygotic twins discordant for BWS, we were able to assess lymphoblasts from 10 twin pairs (9 female, 1 male), all of which showed alterations in KvDMR1 methylation and/ or KCNQ10T1 allelic transcription. Given that MZ twin pairs may share their fetal circulation, we also examined fibroblasts available from 5 twin pairs (4 female, 1 male), and showed that in each pair, the affected twin exhibited loss of maternal methylation at KvDMR1 and/or biallelic expression of KCNQ10T1, whereas the unaffected twin maintained normal methylation of KvDMR1 and monoallelic expression of KCNQ10T1. This consistent difference in KvDMR1/KCNQ10T1 status in these twin pairs suggests that there may be a relationship between postzygotic epigenetic alteration in the imprinted 11p15 region and monozygotic twinning. Studies of X inactivation are underway.

**Association of Tetralogy of Fallot with a distinct region of del22q11.2.** A. Frisch<sup>1,4</sup>, E. Birk<sup>2,4</sup>, A.Y. Weintraub<sup>1,4</sup>, Y. Barhum<sup>1,4</sup>, V. Kotlyar<sup>1,4</sup>, H. Schlesinger<sup>1,4</sup>, R. Rockah<sup>1,4</sup>, B.A. Vidne<sup>3,4</sup>, G. Kessler-Icekson<sup>1,4</sup>. 1) Felsenstein Medical Research C, Tel Aviv University, Petah Tikva, Petah-Tikva, Israel; 2) Pediatric Cardiology, Schneider Childrens Medical Center of Israel; 3) Cardiothoracic Surgery, Beilinson Hospital; 4) Rabin Medical Center, Petach-Tikva, Israel.

Congenital heart defects (CHD) appear in greater frequency among relatives of patients and in individuals with DiGeorge and velo-cardio-facial syndromes (DGS/VCFS). A majority of DGS/VCFS patients and part of the patients with conotruncal heart defects manifest hemizygous deletions within chromosome 22q11.2 (del22q11). We tested the frequency of del22q11 in myocardial tissues of 31 CHD patients, 21 with Tetralogy of Fallot (TOF) and 10 with double chamber right ventricle (DCRV). DNA isolated from tissues removed at corrective surgery was analyzed for homo- or heterozygosity of 9 polymorphic short tandem repeat markers along the 22q11.2 region. DNA from the blood of 45 healthy individuals represented the general population. Homozygosity for 3 consecutive markers was considered an indication for hemizyosity and deletion. Ten out of the 21 TOF patients (48%) showed an indication for deletions of various sizes. No such indication was found for DCRV patients. Heterozygosity for markers D22S1648, D22S941, D22S944 was lower in the TOF group compared with normal controls defining a minimal critical region for the deletion. Our findings support an association between TOF and hemizyosity in 22q11.2, suggesting a distinct region, between markers D22S1638 and COMT, that may harbor TOF susceptibility genes.

**Variations In COL6A1 Coding Region And Congenital Heart Defects In Down Syndrome.** *U.L. Fairbrother, M.J. Baptista, G.E. Davies, D. Trikka, A.M. Kessling.* Medical and Community Genetics, Imperial College, London, Middex, UK.

The pathology of free trisomy 21 cannot ensue simply from overexpression since all individuals with Down Syndrome (DS) do not have a congenital heart defects (CHD). Phenotypic variation is likely to be related to specific alleles, and their protein products, which function well in the diploid but not in the trisomic state. Genetic studies, using RFLP analysis, have implicated the COL6A1-A2 gene cluster at 21q22.3 in CHDs through genotype and haplotype analysis. Notably, in partial trisomy and individuals with DS and a CHD, COL6AI has never been excluded from a causal role. COL6A1 has 36 exons, 30 of which have been screened by single stranded conformational polymorphism (SSCP) analysis in 97 families, including grandparents, parents, individuals with DS with and without CHDs and siblings. Of the individuals with DS, 19 had complete AV canal defect or atrioventricular septal defect (AVSD), 11 had ventricular septal defect (VSD), 5 had atrial septal defect (ASD), 4 had ASD and VSD, 3 had tetralogy of Fallot (ToF), 3 had AVSD and ToF and 4 had other defects. We have shown, for the first time, that there is a higher than expected level of variation in the COL6A1 coding region. In all, 22 variable amplicons have been identified. Mutations have been detected in exons encoding the N-terminal and triple-helical (TH) region, with the most variable region being in exons encoding the C-terminal globular region of collagen VI  $\alpha 1$  chain. We have identified a G to T donor splice site mutation in the triple helical exon 6 which would lead to the addition of 17 amino acids and then premature truncation of the protein product. A parent is heterozygous for this mutation whereas a child, who has received two copies, has AVSD. Alteration and truncation of the TH domain is likely to interfere with helix formation and mutations in the C-ter globular domain will affect chain-chain recognition. We hypothesise that combinations of mutations embedded in particular COL6A1 haplotypes, in trisomic individuals, disturb the supramolecular structure of collagen VI and modulate the predisposition of an individual with DS to a single or several types of CHD.

**Analysis of genetic polymorphisms in the TGFb1, CYP11B2, APOE and AT1R genes in the pathogenesis of acute myocardial infarction at young age and their prognostic role.** S. Carturan<sup>1</sup>, S. Guarrera<sup>1</sup>, A. Gagnor<sup>2</sup>, C. Rolfo<sup>2</sup>, C. Di Gaetano<sup>1</sup>, A. Piazza<sup>1</sup>, GP. Trevi<sup>2</sup>, S. Bergerone<sup>2</sup>, G. Matullo<sup>1</sup>. 1) Dip. Genetica, Biol e Bioch, Università di Torino, Torino, Italy; 2) Divisione di Cardiologia Universitaria, Torino, Italy.

The aim of our study is to evaluate the role both as predisposing and/or prognostic determinants of IMA of polymorphisms in the following genes: Transforming Growth Factor b1 (TGFb1), Aldosterone Synthase (CYP11B2), Apolipoprotein E (Apo E) and Angiotensin I Type 1 Receptor (AT1R). We genotyped a large cohort of subjects with IMA occurred at age <45 years (174 cases, 90.2% males, mean age 39 years) and healthy controls matched by age and sex. Among cases, a subset of 94 patients with follow-up >4 years (mean=7.27±3.31) has been selected to investigate the role of the polymorphisms in the post-IMA decourse: 47 cases had a second or more events (9 re-IMA, 23 PTCA, 20 CABG, 4 deaths). A statistically significant association has been found between IMA at young age and CAD familiarity (p<0.001), smoking (p<0.001), hypercholesterolemia (p=0.026), hypertriglyceridemia (p<0.001) and hypertension (p<0.001). The prevalence of the CC genotype of CYP11B2 gene was 28.7% in the cases vs 17.2% in controls (p=0.040, OR=1.56 95%CI 1.10-2.20); the prevalence of the GG genotype of TGFb1 gene was 28.1% in the cases vs 12.8% in controls (p=0.002, OR=1.94 95%CI 1.33-2.81). In the >4 years follow-up group, a statistically significant increase in the frequency of the Apo E e4 allele has been found among the cases who experienced a second event (e4 carriers vs non-e4 p=0.020, OR=3.59 95%CI 1.40-9.19). A significant increase in the frequency of the C allele of AT1R gene has also been observed in the same group (AC+CC vs AA p=0.017, OR=2.75 95%CI 1.36-5.56). No significant association has been found between CYP11B2 and TGFb1 polymorphisms and a worst prognosis. Our results suggest that the GG genotype of TGFb1 gene and the CC genotype of CYP11B2 gene should be regarded as risk factors for the occurrence of IMA at young age, while the presence of the Apo E e4 allele or AT1R C allele may be associated to a worst prognosis after IMA has occurred.

**Gene Expressions in Apoptosis pathways under Hypothermia conditions can reverse Pathophysiological process of Rabbit Myocardial ischemia.** *S.-H. Chen<sup>1,2</sup>, X.-H. Ning<sup>1,2</sup>, M.A. Portman<sup>1,2</sup>*. 1) Department of Pediatrics, University of Washington, Seattle, WA; 2) Children's Hospital and Regional Medical Center, Seattle, WA.

Functional deterioration of a heart is evident during myocardial ischemia, and can apparently be reversed by hypothermia (<30° C) treatment. The molecular mechanism of this reversal is unknown. We have chosen an isolated heart model to study gene expression under ischemic and hypothermic conditions. Twenty-nine rabbit hearts were divided into a control group (N, n=5), an ischemic group (I, n=12) and a hypothermia group (H, n=12). Functional parameters of the experimental hearts were measured by left ventricular developed pressure (DP), the product of DP and heart rate (PRP), the maximum of the first derivative of DP (dP/dtmax), coronary flow (CF) and oxygen consumption (MVO<sub>2</sub>). Hearts in the hypothermia group showed significant improvement over the ischemic group, after 45 min of re-perfusion. Recovery rates were 37±4 (DP), 32±4 (dP/dtmax), 33±4 (PRP), 57±4 (CF), and 41±4 (MVO<sub>2</sub>) for the I group, and 89±3, 94±4, 88, 92±6, and 92±4 for the H group. Analyses of cDNA Arrays (Clontech Laboratory Inc. and Super Array Inc) identified 14 genes that showed significant changes in expression (greater than 2 to less than 0.5 fold) in the hypothermia group compared to the ischemic or control group. Seven of the 14 genes were related to apoptosis pathways: Tumor suppressor and apoptosis gene (p53), Growth-arrest and DNA damage-inducible transcript 1 (GADD45), caspase 10 (mch4), p21, mad3, bak and bcl-x. Expression of the major apoptosis gene p53 was further investigated by a quantitative RT-PCR method. In the ischemic heart, p53 expression was found to increase 4.7 fold and in the hypothermic heart, p53 expression was about 0.7 fold of the controls. Change in gene expression in the hypothermic group was always accompanied by an excellent functional recovery of the heart. This data indicates that under ischemic condition, hypothermia is protective of myocardial damage by preventing over-expression of key apoptosis genes.

**Genetic susceptibility to preeclampsia: evaluation of eNos , TGFB3, and AGT mutations.** *D.M. Caprau<sup>1</sup>, J.C. Murray<sup>1</sup>, H.L. Mertz<sup>2</sup>, Y. Kim<sup>1</sup>, D.C. Merrill<sup>2</sup>.* 1) Dept of Pediatrics University of Iowa, Iowa City, IA; 2) Wake Forest University, Winston-Salem, NC.

Genetic Susceptibility to Preeclampsia: Evaluation of NOS, TGFB3, and AGT Mutations. D. Caprau, J.C. Murray, H.L. Mertz, Y. Kim, D.C. Merrill Objective: Previous studies have suggested an association of preeclampsia (PRE) with several genes involved in cardiovascular control. The objective of this study was to evaluate the association between PRE and allelic variants of eNOS, TGFB3 and Angiotensinogen (AGT) genes in Caucasian women. Methods: Samples were extracted from whole blood or buccal cells of preeclamptic patients and controls. PRE was diagnosed using standard criteria (new onset hypertension and proteinuria). Controls (CON) consisted of women who had undergone at least two term pregnancies unaffected by PRE. All samples were genotyped for each polymorphism using PCR followed by SSCP analysis. Results: The 27 bp repeat intron four of eNOS was typed in 1,001 samples (680 controls and 321 cases). Heterozygous and homozygous carriers of the eNOS 27 bp repeat mutation were observed with similar frequencies in CON and PRE subjects (homozygous: CON=2.8%; PRE=2.8%; heterozygous: CON=23.4%; PRE=22.4%,  $p=0.94$ ). For TGFB3, the CA repeat at 14q24 was analyzed on 713 samples (299 cases and 414 controls). No significant association between PRE and this loci was observed ( $p=0.28$ ). Five hundred sixty-three samples (272 controls and 291 cases) were genotyped for the A(-6)G mutation of the AGT gene. 5 of 272 (1.8%) of controls versus 13 of 291 (4.5%) of PRE subjects were homozygous for this mutation ( $p=0.0764$ ). Overall 85 of 272 (31.3%) of CON versus 112 of 291 (38.5%) of PRE subjects were carriers of this mutation ( $p=0.072$ ). Conclusion: These data suggest a possible association of a mutation in the AGT gene and susceptibility to PRE. Further sample analysis is underway to confirm these preliminary results.



**Variable Cardiac Phenotypes in Chromosome 4q- Syndrome with dHAND Deletion.** *T. Huang*<sup>1</sup>, *S. Weremowicz*<sup>2</sup>, *G. Cox*<sup>1</sup>, *A.E. Lin*<sup>3</sup>, *W. Golden*<sup>4</sup>, *G.L. Feldman*<sup>5</sup>, *S. Vermeulen*<sup>6</sup>, *U. Moog*<sup>7</sup>, *C. Schrandt-Stumpel*<sup>7</sup>, *C.C. Morton*<sup>2</sup>, *J.G. Seidman*<sup>8</sup>, *C.E. Seidman*<sup>9</sup>. 1) Div of Gen/Harvard Med School, Children's Hospital, Boston, MA; 2) Departments of Pathology and Obstetrics, Gynecology and Reproductive Biology Brigham and Womens Hospital, Boston; 3) Teratology Program, The Brigham and Womens Hospital; 4) Division of Genetics, University of Virginia, Charlottesville; 5) Department of Medical Genetics, Henry Ford Hospital, Detroit, Michigan; 6) Centrum Medische Genetica, UZG-K5, De Pintelaan; 7) Department of Clinical Genetics University of Limburg; 8) Department of Genetics, Howard Hughes Medical Institute, Harvard Medical School; 9) Department of Cardiology, The Brigham and Womens Hospital, Howard Hughes Medical Institute, Boston.

Terminal deletions of chromosome 4q are commonly associated with cardiovascular malformations (CVMs). The dHAND gene, a basic helix-loop-helix transcription factor expressed in the developing heart, has been mapped to 4q33. A targeted deletion mouse showed that dHAND plays an important role in heart development. To examine the association of dHAND haploinsufficiency with the CVMs which occur in some patients with 4q terminal deletions, we performed fluorescence in situ hybridization (FISH) with a dHAND genomic probe on 5 patients. Of the three with a deletion of the dHAND locus, two had a CVM (both valvar pulmonic stenosis). Of the two patients without a deletion of the dHAND gene, one had a small atrial septal defect noted on autopsy. In this small series of terminal 4q deletions, haploinsufficiency of the dHAND seems not to correlate well with a CVM. This may be explained by low penetrance of the d-HAND gene or other factor(s) which contribute to the development of the CVMs.

**Molecular characterization of deletions, mutations and meiotic recombination within the genomic region 22q11 in patients with congenital heart defects.** *J. Rosell<sup>1,3</sup>, L. Torres-Juan<sup>1</sup>, N. Govea<sup>1</sup>, M.A. de la Fuente<sup>2</sup>, F. García-Algas<sup>2</sup>, D. Heine-Suñer<sup>1,3</sup>.* 1) Genetics Department, University Hospital Son Dureta, Palma de Mallorca, Balearic Islands, Spain; 2) Paediatrics Department, University Hospital Son Dureta, Palma de Mallorca, Balearic Islands, Spain; 3) Biology Department, University of the Balearic Islands, Palma de Mallorca, Balearic Islands, Spain.

De novo deletions in the 22q11 genomic region have an estimated prevalence at birth of 1/600-1/4000 according to different authors. Most patients have the same 3 Mb deletion but show a complex and variable phenotype that ranges from a DiGeorge syndrome to isolated congenital heart disease. There are two not completely solved issues in this complex disease: 1) is the absence of genotype-phenotype correlation and 2) what are the molecular mechanisms that result in such a high incidence of de novo deletions. To try and answer such questions in an unbiased manner we have performed FISH analysis on patients with conotruncal heart defects (the most frequent clinical feature found in patients with the 22q11 deletion, 75%). Such analysis detected deletions in 8/55 subjects (14.5%), which is similar to that reported in other studies. We then used 17 polymorphic markers to further characterise these deletions and detect other atypical deletions by loss of heterozygosity. Such analysis determined that most patients carried the typical 3 Mb deletions and that these had originated de novo. We also found that 4 deletions affect the maternal chromosome and 1 the paternal chromosome. Recently, mutations in the *Tbx1* gene which maps to the 22q11 region, have been shown to cause conotruncal heart defects in mice. SSCP analysis of this gene in non-deleted patients has been unsuccessful so far in detecting any mutations. Phenotype-genotype correlation has also been examined in an effort to delineate clinical differences that may distinguish deleted and non-deleted patients. Finally, we have constructed a high resolution genetic map that outlines the recombinational characteristics of the 22q11 region and which may help explain the high rate of deletions observed in this disease.

**Family based investigation of the role of a polymorphism in the paraoxonase (PON) 1 gene in ischaemic heart disease (IHD).** *M.S. Spence<sup>1</sup>, P.G. McGlinchey<sup>1</sup>, C. Patterson<sup>2</sup>, C. Belton<sup>3</sup>, G. Murphy<sup>1</sup>, D. McMaster<sup>3</sup>, D. Fogarty<sup>4</sup>, P.P. McKeown<sup>1, 3</sup>.* 1) Regional Medical Cardiology Centre, Royal Victoria Hospital, Belfast, Northern Ireland, UK; 2) Department of Epidemiology and Public Health, Queen's University, Belfast; 3) Department of Medicine, Queen's University, Belfast; 4) Antrim Area Hospital, Northern Ireland.

**Background:** IHD is a complex phenotype arising from the interaction of genetic and environmental factors. Oxidative modification of low-density lipoprotein (LDL) is currently thought to be central to the pathogenesis of IHD. Paraoxonase (PON1) is an HDL associated enzyme that protects LDL from oxidative modification. A polymorphism at codon 192 of the PON1 gene results in a glutamine (Q) to arginine (R) substitution and is associated with altered paraoxonase activity. Several, but not all case-control studies have implicated this polymorphism with the risk of IHD. Given these conflicting results we have used a family based association test to investigate the presence of linkage disequilibrium between the codon 192 PON1 polymorphism and IHD in a well-defined Irish population.

**Methods:** A total of 157 families were recruited (418 individuals). The presence of linkage disequilibrium between the codon 192 PON1 polymorphism and IHD was investigated using the combined transmission disequilibrium test (TDT) / sib TDT. This test is unaffected by population admixture and was specifically designed for the investigation of complex diseases. Only one discordant sibship or trio was included from each family to ensure the analysis was a valid test of association. We tested for excess transmission of the Q or R allele to affected individuals.

**Results:** 50 of 134 discordant sibships and 20 of 25 trios (proband and both parents) were informative for the analysis. There was no statistically significant excess transmission of either allele to affected individuals, ( $p=0.60$ ).

**Conclusion:** using a recently developed family-based association test we found no evidence that the codon 192 polymorphism in the PON1 gene is associated with IHD, in our study population.

**Evidence that interaction occurs among genes regulating plasma levels of apolipoprotein A2.** *L.J. Raffel<sup>1,2</sup>, L.W. Castellani<sup>2</sup>, L.S-C. Cheng<sup>1,2</sup>, R.C. Davis<sup>2</sup>, M. Quinones<sup>2</sup>, A.H. Xiang<sup>3</sup>, P-Z. Wen<sup>2</sup>, J. Diaz<sup>3</sup>, W.A. Hsueh<sup>2</sup>, T.A. Buchanan<sup>3</sup>, J.I. Rotter<sup>1,2</sup>.* 1) Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) UCLA School of Medicine, Los Angeles, CA; 3) USC Keck School of Medicine, Los Angeles, CA.

Apolipoprotein A2 (ApoA2) is the second most abundant protein present in HDL. While elevated plasma ApoA2 levels are associated with obesity, diabetes and increased plasma free fatty acids, no mechanism for this association is apparent. Transgenic animal studies have shown that elevated ApoA2 increases weight, plasma free fatty acids, leptin, and insulin.

As part of a study of the genetics of hypertension and insulin resistance, a 10 cM genome scan has been completed in 390 members of 77 Hispanic families ascertained via hypertensive probands. We have observed loci on chr. 1, 2, 3, 6 and 7 that show linkage with plasma ApoA2 levels. To further investigate these observations, we performed additional analyses, including oligogenic analysis and 2 locus-joint effects using SOLAR. Oligogenic analysis was done conditioning on the chr. 6 locus (69 cM), which had the largest single locus LOD score (3.61). Evidence for linkage remained significant for loci on chr. 1 (214 cM, LOD 1.42,  $p=0.01$ ), 3 (7 cM, LOD 1.77,  $p<0.005$ ), and 7 (79 cM, LOD 1.5,  $p<0.009$ ). Linkage to chr. 2 was no longer significant. There is also evidence for interaction among these loci in determining ApoA2 levels, with the following joint LOD scores: chr.6-chr.3, 5.38 ( $p=0.000001$ ); chr.6-chr.7, 5.11 ( $p=0.000003$ ); chr.6-chr.1, 5.03 ( $p=0.000003$ ); chr.1-chr.3, 5.08 ( $p=0.000003$ ); chr.1-chr.7, 4.31 ( $p=0.000017$ ); chr.3-chr.7, 4.57 ( $p=0.000009$ ).

These results suggest that ApoA2 levels are regulated by at least 4 loci and that these loci act synergistically. The locus on chr. 1 is in the region of the ApoA2 structural gene, but possible candidate genes for the chr. 3, 6 and 7 loci remain to be identified. Further analyses of these families, including fine mapping in the peak linkage regions, should allow us to better localize the underlying genes and perhaps identify candidates that regulate this important phenotype.

**ACE and AGT polymorphisms in ischaemic stroke and carotid stenosis: association and sibs study. T.V.**

*Tupitsina<sup>1</sup>, O.V. Miloserdova<sup>1</sup>, P.A. Slominsky<sup>1</sup>, Eu.A. Koltsova<sup>2</sup>, V.I. Skvortsova<sup>2</sup>, S.A. Limborska<sup>2</sup>.* 1) Inst. of Molecular Genetics, Moscow, Russia; 2) Russian State Medical Academy, Moscow, Russia.

Family and twins study demonstrated that genetic factors may be involved in stroke. Previously, insertion/deletion (I/D) Alu-polymorphism in the angiotensin-converting enzyme (ACE) gene and M235T polymorphism in the angiotensinogen gene has been suggested as a risk factors for some cardiovascular diseases. There fore, cardiovascular factors are well-known risk factors for ischaemic cerebrovascular disease (ICVD). We have investigated the distribution of alleles of ACE and angiotensinogen genes in group of patients with ischaemic and their sibs. All patients and sibs with ischaemic stroke were investigated for the stenosis of the internal carotid artery (CS). There was a significant difference ( $P < 0.05$ ) in the distribution of ACE alleles, homozygosity for the presumed susceptibility deletion allele being more common in patients with profound (>50%) CS than in healthy control subjects and healthy sibs. There was also a significant difference ( $P < 0.05$ ) in patients with CS in comparison with matched ICVD patients without CS, both in allelic frequencies and in homozygosity for the deletion allele. However, ID allele distribution demonstrated no evidence for statistically significant differences in frequencies of I/D alleles or II/DD/ID genotypes between ICVD patients and healthy control subjects. Our results indicate that the ACE gene polymorphism may be a risk factor for the development of CS - but not for ICVD. M235T angiotensinogen gene polymorphism is not risk factor for ICVD or CS in our patient's group - we don't find any significant differences in allele and genotype distribution between ICVD and CS patients and healthy control subjects.

**Inverse regulation of LDL receptor-related protein (LRP) on transcriptional and translational level in patients with CAD.** *S. Schulz<sup>1</sup>, U. Schagdarsurengin<sup>1</sup>, P. Greiser<sup>1</sup>, G. Birkenmeier<sup>2</sup>, L. Weidhase<sup>3</sup>, A. Nordwig<sup>3</sup>, A. Kabisch<sup>4</sup>, K. Werdan<sup>3</sup>, I. Hansmann<sup>1</sup>, C. Gläser<sup>1</sup>.* 1) Inst Human Genetics, Univ Halle, Halle, Germany; 2) Inst Biochemistry, Univ Leipzig, Leipzig, Germany; 3) Dep Internal Med, Univ Halle, Halle, Germany; 4) Inst Transf Med/Bloodbank, Univ Halle, Halle, Germany.

LRP, is a multifunctional cell receptor which binds and internalizes a variety of important ligands and is therefore considered to be a candidate gene for degenerative diseases like atherosclerosis and Morbus Alzheimer. **Material and Methods:** We investigated the individual LRP-mRNA and protein expression in native monocytes from 36 male patients with angiographically proven severe coronary atherosclerosis (average age: 51.7 years) in contrast to 36 healthy long-standing male blood donors (average age: 47.3 years). The investigations on transcriptional level were carried out by means of a competitive RT-PCR. For specific detection of LRP protein expression we applied a macro array analysis using a commercially available LRP standard protein of human placenta of known concentration as a reference.

**Results:** We measured a significantly 1.82 fold higher LRP-mRNA expression in coronary patients in comparison to healthy controls (223 ag/cell vs. 122.3 ag/cell,  $p < 0.001$ ). However the investigation of LRP-protein expression revealed an inverse pattern: Whereas the expression of the coronary patients amounted to 1.6 pg/cell the controls showed a significant higher protein expression of 6 pg/cell ( $p < 0.001$ ). Obviously a high LRP-mRNA expression was associated with a low protein expression, and vice versa. These results suggest a complex regulatory mechanism of the LRP at transcriptional and translational level. The detected lower protein expression in coronary patients may be due to a severe unbalanced metabolism in atherosclerosis. This could lead to a diminished receptor-mediated endocytotic pathway in coronary patients which may then be compensated by increasing the mRNA expression. These findings supply evidence for the importance of the expression pattern of the receptor in the assessment of atherosclerosis development.

**Association between hypertension, AGT, DCP1, and LPL genes, and environmental factors: results of Nutrition and Health Survey in Taiwan, 1993-1996.** *W.H. Pan<sup>1</sup>, P.J. Shieh<sup>1</sup>, H.Y. Chang<sup>2</sup>, C.S.J. Fann<sup>1</sup>*. 1) Inst of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, Taiwan; 2) National Health Research Institute, Taipei, Taiwan.

Linkage between *angiotensinogen (AGT)*, *angiotensin converting enzyme (DCP1)* and hypertension has been repeatedly studied. Our previous study has also indicated that markers of *lipoprotein lipase (LPL)* and *DCP1* may be linked to young-onset hypertension in Taiwanese. Using the DNA samples collected by the Nutrition and Health Survey in Taiwan (NAHSIT), we carried out an association study on hypertension and *AGT*, *DCP1*, and *LPL* genes. We randomly selected 208 hypertensive subjects (96 subjects aged < 40 and 112 subjects aged 40+) and 248 age, sex, and region matched controls from the NAHSIT database. Genotyping was carried out for *DCP1 D/I* polymorphism, *AGT M235T* and *AGT T174M* polymorphism, and for microsatellite markers of *DCP1 (ATA108a05)* and of *LPL*. Nutrient intakes of the previous 24-hours, anthropometric measurements, and clinical chemistry profiles were also included as environmental factors in the analysis. Hypertensive men and women tend to be more obese, had higher level of serum triglyceride, uric acid, and glucose, and drank more alcohol (in men only) than their normotensive counterparts. No association was found between hypertension and any gene. But presence of M allele of *AGT T174M* and allele 4 (with 157 AAT repeats) of the *ATA108a05* was associated with an odds ratio of 5.65 (1.4-22.9) for hypertension risk. Gene-environmental interaction was found between *AGT M235T* polymorphism and body mass index and between *DCP1 D/I* polymorphism and the ratio of dietary polyunsaturated fatty acid to saturated fatty acids. In addition, a clear dose-response relation was observed between D allele of *DCP1* and serum triglyceride level in men. These data indicate that genes and environmental factors interact in their contributions to the development of hypertension.

**C-260T polymorphism in the promoter region of the CD14 receptor gene is associated with pro-inflammatory response in patients with unstable angina.** *P.F. Pignatti<sup>1</sup>, C. Stranieri<sup>1</sup>, G. Liuzzo<sup>2</sup>, E. Trabetti<sup>1</sup>, C. Alongi<sup>1</sup>, D.J. Angiolillo<sup>2</sup>, M. Santamaria<sup>2</sup>, V. Rizzello<sup>2</sup>, C. Colizzi<sup>2</sup>, F. Ginnetti<sup>2</sup>, A. Buffon<sup>2</sup>, L.M. Biasucci<sup>2</sup>, A. Maseri<sup>2</sup>.* 1) Sect Biol & Genetics, Mother & Child, Biol & Gen, Verona, Italy; 2) Cardiology, Catholic University, Rome, Italy.

The CD14 membrane receptor is an important mediator for monocytes (MO) activation by bacterial lipopolysaccharides (LPS). In this study, we examined whether the C-260 T polymorphism in the CD14 gene influences the activation of circulating MO and the acute phase response in unstable angina (UA). CD14 C-260 T polymorphism, plasma levels of C-reactive protein (CRP), and interleukin-6 (IL-6) production were determined in 87 UA patients (Group 1A, 52 patients with CRP  $\geq$  3 mg/L; Group 1B, 35 patients with CRP  $<$  3 mg/L), in 39 stable angina patients (Group 2), and in 68 healthy subjects (Group 3). LPS-stimulated production of IL-6 by circulating MO was significantly higher in Group 1A (4.4 ng/mL, 2.1-10.6 ng/mL) than in Group 1B (1.9 ng/mL, 0.7-6.8 ng/mL) or in Group 2 (1.4 ng/mL, 0.4-6.3 ng/mL) ( $p < 0.01$ ). The TT genotype was present in 23/52 (44%) Group 1A, in 4/35 (11%) Group 1B, in 7/39 (18%) Group 2 patients, and in 14/68 (20%) Group 3 controls, a significant difference in TT genotype distribution between Group 1A and Groups 1B and 2 ( $p = 0.001$ ) or Group 3 ( $p = 0.02$ ). TT homozygotes showed an increased production of IL-6 by circulating MO in response to LPS-challenge (4.1 ng/mL, 1.6-10.6 ng/mL) than carriers of the other two genotypes (2.5 ng/mL, 0.24-6.3 and 3.1 ng/mL, 0.9-9.6 ng/mL, respectively;  $p < 0.01$ ). TT homozygotes had significantly higher CRP plasma levels (5.9 mg/L, 0.6-43.4 mg/L) than carriers of both the CC (1.7 mg/L, 0.8-29.7 mg/L) and CT (2.7 mg/L, 0.1-29.4 mg/L) genotypes ( $p < 0.05$ ). In conclusion, the C-260T polymorphism in the promoter region of the CD14 receptor gene is associated with UA and is correlated with an enhanced responsiveness of circulating MO after LPS-challenge in UA, suggesting that the inflammatory response in UA may be genetically determined.



**Somatic mutations in sporadic juvenile hemangioma.** *J.W. Walter<sup>1</sup>, P.E. North<sup>2</sup>, A. Mizeracki<sup>2</sup>, M. Waner<sup>2</sup>, J.F. Reinisch<sup>3</sup>, J. Walker<sup>3</sup>, F. Blei<sup>4</sup>, C. Patterson<sup>5</sup>, D.A. Marchuk<sup>1</sup>.* 1) Duke Univ Medical Ctr, Durham NC; 2) Univ Arkansas, Little Rock AR; 3) Childrens Hospital, Los Angeles CA; 4) NYU Medical Ctr, New York NY; 5) Univ North Carolina, Chapel Hill NC.

Juvenile hemangioma is the most common tumor of infancy, and occurs in as many as 10% of all births. These benign vascular lesions enlarge rapidly during the first year of life by hyperplasia of endothelial cells and attendant pericytes, then spontaneously involute over a period of years, accumulating fibrofatty tissue. At least three distinct hypotheses have been proposed to account for hemangioma formation: (1) The proliferative vascular cells are derived from a distinct anatomical location (e.g., placental origin hypothesis). (2) Normal vascular cells are responding to aberrant signal from adjacent tissue (e.g., extrinsic growth factors). (3) The vascular cells from the lesion are intrinsically altered (e.g., somatic mutation).

To test this latter hypothesis, we obtained 15 proliferative hemangiomas that had been surgically resected, and dissected to enrich for the proliferative component of the lesions. 12 of 15 hemangiomas showed evidence of monoclonality, using X-inactivation assays at the HUMARA locus. Control tissue from overlying skin showed no evidence of monoclonality, suggesting an intrinsic defect in the proliferative endothelial cells. Genes involved in angiogenesis were sequenced in these samples. Two lesions were found to harbor mutations in VEGF receptors resulting in amino acid substitutions: P1147S in VEGFR2, and P954S in VEGFR3. Both mutations occur somatically, as neither is found in adjacent normal tissue. Both proline to serine substitutions occur in the kinase regions of these receptors, and preliminary biochemical studies of the VEGFR2 mutation suggest a loss of kinase activity. We propose that multiple independent events can cause hemangiomas, as suggested by the high incidence of these tumors in the population. The identification of somatic mutation of VEGF receptors in a subset of these tumors is an important step toward understanding the molecular etiology of hemangiomas.

**Clonal analysis of endometriotic lesions using laser capture microdissection and PCR-based HUMARA assay. S.**

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Endometriosis is a common gynecological disorder that generally follows a benign course, yet displays characteristics similar to malignant neoplasia. Several recent clonality studies suggested that the majority of endometriotic lesions are monoclonal in origin. In these studies, the clonality of epithelial cells was evaluated based on a polymorphic trinucleotide CAG repeat marker of the X-linked human androgen receptor gene HUMARA. With this approach, only the inactivated (methylated) HUMARA allele is selectively amplified by PCR following methylation-sensitive enzyme digestion and therefore a methylation pattern can be established. To minimize the chance of contamination that could lead to spurious clonal determination, we used laser capture microdissection (LCM) to accurately sample individual epithelial cells from endometriotic lesions and control samples out of frozen tissues and hence determine their clonality with greater certainty. Three samples of endometriotic lesions and their corresponding controls from three patients were analyzed. All of the control samples showed heterozygous for the CAG repeat polymorphism and hence were polyclonal in origin. The epithelial cells in two endometriotic lesion samples on the other hand showed monoclonal patterns. Our results corroborate previous reports on the clonality of endometriotic lesions. Since the use of LCM allows accurate and precise tissue sampling from small endometriotic lesions, our results brought more certainty that endometriotic lesions are monoclonal in origin and may thus carry a neoplastic potential.

**A female with Fragile-X shows an affected male-like phenotype and skewed inactivation of the functional chromosome.** *D. Heine-Suñer<sup>1,4</sup>, G. Picó<sup>2</sup>, L. Torres-Juan<sup>1</sup>, M. Bernués<sup>1</sup>, J. Iglesias<sup>3</sup>, F. Barceló<sup>1</sup>, J. Rosell<sup>1,4</sup>.* 1) Department of Genetics, University Hospital Son Dureta, Palma de Mallorca, Balearic Islands, Spain; 2) Department of Paediatrics, University Hospital Son Dureta, Palma de Mallorca, Balearic Islands, Spain; 3) Department of Immunology, University Hospital Son Dureta, Palma de Mallorca, Balearic Islands, Spain; 4) Department of Biology, University of the Balearic Islands, Palma de Mallorca, Balearic Islands, Spain.

Fragile X syndrome is the most common form of inherited mental retardation. It is caused by the increase in length of a stretch of CGG triplet repeats within the FMR1 gene. A full mutation (>200 repeats) leads to methylation of the CpG island and silencing of the FMR1 gene. We present here a family with two young sisters (11 and 16 years old) that are compound heterozygotes for the full mutation and a 53 repeat allele (premutation grey zone), one of them showing mental retardation and clinical features of an affected male (speech delay, hyperactivity, large ears, prominent jaw, gaze aversion), while the other is borderline normal (mild delay). Southern blot analysis to detect methylation status showed that the sister with mental retardation had practically all her cells (skin and blood) with the normal FMR1 gene inactivated, while her sister had most of her blood cells with the mutated FMR1 gene inactivated. Furthermore, skewed X-inactivation of the non-functional chromosome was also observed in another affected, mildly retarded female member of the family. Analysis of X-inactivation in the extended family with the Androgen Receptor (AR) inactivation assay confirmed a highly skewed X-inactivation pattern in the two sisters and other members of the family. Molecular analysis by PCR of family members carrying the 53 repeat allele showed that it is inherited in a stable manner and thus, does not behave like a premutation. Further investigations are under way to confirm that the peculiar phenotype observed in one sister is caused by an extremely skewed inactivation of the functional chromosome. ñññ.

**Temperament and Character Profiles of ADHD and Subtypes.** *D.E. Lynn, J.R. McCracken, J.J. McGough, G. Lubke, A. Liu, S. Smalley.* Child and Adolescent Psychiatry, UCLA, 300 Medical Plaza LA, CA.

Objective: Our study was designed to investigate the link between Attention Deficit Hyperactivity Disorder (ADHD) in adults, Novelty Seeking (NS) temperament scores on the Temperament Character Inventory (TCI), and genotype at polymorphisms in the D4 Dopamine Receptor (DRD4) Gene. Method: Data from 171 parents from 96 families were used. This study draws from a larger molecular genetic study of ADHD, in which ascertainment criteria was to have at least two children affected (an Affected Sib Pair, ASP) with ADHD in each family. Of these 171 parents, 56 (33%) parents had a lifetime history of ADHD, of these 25 (12%) continued to meet DSM-IV criteria (i.e. persistent ADHD). 115 (67%) of these showed no history of ADHD. Latent variable modeling and analysis of variables (ANOVA) were used to test whether TCI factors could predict ADHD outcome measures. Results: Using Latent Variable Modeling, we were able to confirm the first order factor of the TCI. We found that NS predicted ADHD Diagnosis ( $R^2 = .28$ ). Using ANOVA, we found elevated NS is associated with a lifetime history of ADHD ( $p < .01$ ). Character measures from the TCI predict subtype of ADHD ( $p < .05$ ). There was no association found between the DRD4 gene and TCI measures in this sample. Conclusions: In this unique sample of parents of multiplex families, NS is a potential risk factor for development of ADHD. Genetic influences underlying temperament may contribute to the etiology of ADHD and character may be a mediator of the variation in outcomes of ADHD.

**Subtelomeric plasticity: the FSHD enigma.** *R.J.L.F. Lemmers<sup>1</sup>, P. de Kievit<sup>1</sup>, M.J.R. van der Wielen<sup>1</sup>, E. Bakker<sup>1</sup>, G.J. van Ommen<sup>1</sup>, G.W. Padberg<sup>2</sup>, R.R. Frants<sup>1</sup>, S.M. van der Maarel<sup>1</sup>.* 1) Human & Clinical Genetics, LUMC Leiden, Leiden, Netherlands; 2) Dept. of Neurology, UMCN Nijmegen, Nijmegen, The Netherlands.

Subtelomeres are dynamic structures as exemplified by the recombinogenic nature of the D4Z4 repeat on chromosome 4qter. Rearrangements of this subtelomeric repeat array are not only confined to chromosome 4, but may also occur between chromosome 4 and a highly homologous repeat on chromosome 10qter. As a result, (partial) D4Z4 repeats are frequently observed on chromosome 10. Likewise, homologous units from chromosome 10 may also be present on chromosome 4.

Partial deletions of the D4Z4 repeat on chromosome 4 cause autosomal dominant facioscapulohumeral muscular dystrophy (FSHD). Partial deletions on chromosome 10 are non-pathogenic. The extensive clinical variability of FSHD, the high *de novo* mutation frequency, and the clinical overlap with other syndromes necessitates a highly specific genetic test. Yet, the dynamic behavior of these subtelomeric repeats compromises FSHD diagnosis. Genetic diagnosis is based on sizing of the D4Z4 repeat array with probe p13E-11, which also recognizes the homologous repeat on chromosome 10. To discriminate between both chromosomes, *BlnI* is used which recognizes chromosome 10 units, but not chromosome 4 units.

To further optimize complete allele assignment of repeat arrays on both chromosomes and to assist the study of subtelomeric plasticity, we introduce the restriction enzyme *XapI*. It displays opposite characteristics of *BlnI* by uniquely digesting 4-type repeat units and leaving 10-type repeat units undigested. Using the combination *EcoRI*, *EcoRI/BlnI* and *XapI* unequivocally allows detailed analysis of the recombinogenic behavior of these subtelomeric domains by complete characterization of each allele, whether homogeneous or hybrid. This is particularly useful in case of co-migrating 4-type and 10-type arrays, for the assignment of hybrid fragments to their original alleles and in case of suspected FSHD with non-standard allele configurations as demonstrated by exclusion of one patient carrying an apparently short hybrid repeat array.

**Paternally inherited deletions of CSH1 in Silver-Russell syndrome.** *T. Eggermann<sup>1</sup>, S. Prager<sup>1</sup>, S. Mergenthaler<sup>1</sup>, K. Eggermann<sup>1</sup>, M.B. Ranke<sup>2</sup>, K. Zerres<sup>1</sup>, H.A. Wollmann<sup>2</sup>.* 1) Inst Human Genetics, Technical Univ Aachen, Aachen, Germany; 2) Childrens Hospital, University of Tuebingen, Germany.

Silver-Russell syndrome (SRS) is a syndrome of severe pre- and postnatal growth retardation and typical dysmorphic features. Rare chromosomal aberrations have been reported in SRS, among these are two balanced translocations involving 17q24-q25. We recently described a patient with a paternally inherited heterozygous deletion of the chorionic somatomammotropin hormone 1 (CSH1) gene. The CSH1 gene is member of the growth hormone (GH) gene cluster in 17q22-24 which consists of two growth hormone genes and three CSH genes. Genomic alterations in the GH cluster are well known, causing different phenotypes depending on the size of the deletion and the genes involved. By screening 62 SRS cases with marker D17S254, we now detected a second patient with a heterozygous deletion in the GH cluster inherited by his father. Quantitative analysis using a PCR-based protocol showed that the patient and his father were hemizygous for CSH1 while the neighboring gene GH2 was not deleted. Screening of 50 unrelated controls did not reveal haploinsufficiency of CSH1. While the central role of GH1 in human growth is well established, the roles of CSH1 and other components of the cluster remain to be investigated. It will be interesting to see, under which circumstances haploinsufficiency of CSH1 causes clinical features. Furthermore, the finding of a second case with CSH1 deletion shows the usefulness of molecular genetic CSH1 testing in SRS patients, which should be added to a routinely performed cytogenetic and uniparental disomy 7 analyses in SRS.

**Absence of prevalent sequence variations in the HSP60 and HSP10 chaperonin genes in 65 cases of Sudden Infant Death Syndrome (SIDS).** *P. Bross<sup>1</sup>, J.J. Hansen<sup>1</sup>, Z. Li<sup>1,2,3</sup>, M. Nyholm<sup>1</sup>, S. Kølvråa<sup>3</sup>, J.B. Lundemose<sup>4</sup>, L. Bolund<sup>3</sup>, H. Yang<sup>2</sup>, N. Gregersen<sup>1</sup>.* 1) Res. Unit for Mol. Med. at Aarhus University Hospital, Århus, Denmark; 2) Chinese Academy of Sciences Inst. for Genome Research, Beijing, China; 3) Inst. of Human Genetics at Aarhus University, Århus, Denmark; 4) Inst. of Forensic Medicine at Aarhus University Hospital, Århus, Denmark.

The Hsp60 chaperonin and the co-chaperonin Hsp10 function together at a strategic point in mitochondrial protein folding. Variations in these chaperonins - that are highly conserved between species - may be expected to have rather dramatic pleiotropic effects by affecting the folding of many mitochondrial proteins. An RFLP study on infants dead in sudden infant death syndrome (SIDS) indicated that a certain RFLP in the Hsp60 gene was overrepresented in this group compared to controls. We have analysed 10 Danish SIDS cases and 10 control individuals by high quality sequencing of both strands in the HSP60 and the HSP10 coding region, the intron sequences proximal to the exon/intron junctions and the bi-directional promoter localised between the genes. In addition, 55 SIDS cases were screened by single run sequencing of the same regions. Altogether, we detected 13 variations in the Hsp60/10 genes. 4 of them are located in the promoter region, 5 in introns and 4 in the Hsp60 coding region. Two of the variations in the coding region alter the amino acid code. One of them (G537A) is present at similar frequency in both the SIDS group and controls. The other amino acid variation (N158S) is only found in one allele of a single SIDS case and is not present in 100 control alleles. None of the 13 sequence variations detected in the HSP60 and HSP10 genes is significantly overrepresented in 65 SIDS infants indicating that mutations in these genes are no prevalent cause of sudden infant death. The variation N158S that is observed in only one SIDS individual may represent a rare disease allele of the HSP60 gene.

**The 11q deletion disorder: A prospective study of 91 cases and identification of a candidate gene for heart defects.** *P.D. Grossfeld<sup>1</sup>, C. Jones<sup>2</sup>, F. Cotter<sup>2</sup>, J. Chen<sup>1</sup>, P. Lozano<sup>1</sup>, L. Zelante<sup>3</sup>, M. Del'Aquilla<sup>1</sup>, F. Parente<sup>1</sup>, K. Chien<sup>1</sup>.* 1) Div of Pediatric Cardiology, Univ University of California, San Diego, San Diego, CA; 2) St. Bartholomew School of Medicine, London, England; 3) Medical Genetics Service, Rotondo, Italy.

Jacobsen Syndrome (JS) is a dysmorphic disorder caused by terminal deletions of 11q. We studied 91 JS cases. These patients commonly have congenital heart disease (CHD), mental retardation (MR) (language and music comprehension deficits), thrombocytopenia, genitourinary defects, short stature, eye problems, craniofacial defects, pyloric stenosis, and chronic constipation. Fifty-four percent of JS children have CHD, most commonly left-sided obstructive lesions and perimembranous ventricular septal defects. Other heart defects seen in JS include conotruncal defects, pulmonary stenosis, double outlet left ventricle, AV canal defect, tricuspid atresia, and transposition of the great arteries. We performed deletion mapping by Fluorescent In Situ Hybridization of 35 JS patients with CHD and identify a minimal region for heart defects in 11q containing ~20 known genes. We also define the molecular breakpoint of a patient with a severe heart defect and demonstrate that the breakpoint interrupts a gene that is within the JS cardiac minimal region. This gene, OBCAM (Opiate-Binding Cell Adhesion Molecule) is a member of the immunoglobulin gene superfamily. We demonstrate that this gene is expressed in a chamber-specific pattern in the heart. We also show that Neurotrimin and Limbic-Associated Membrane Protein (LAMP), two other members of the IgLON subfamily of cell adhesion molecules, are also expressed in a cardiac chamber-specific pattern in the heart. We propose that cell adhesion molecules have a role in cardiac development and that loss of heterozygosity of OBCAM may contribute to the development of congenital heart defects. We have also identified critical minimal regions for the other phenotypes in JS. Minimal regions for pyloric stenosis, genitourinary defects and MR are within the cardiac minimal region, thereby defining a region containing less than 20 genes that may be responsible for each of these defects.



**Microarray analysis of a Smith-Magenis patient.** *C.N. Vlangos<sup>1</sup>, S.H. Elsea<sup>1,2</sup>*. 1) Graduate Program in Genetics, Michigan State University, East Lansing, MI 48824; 2) Departments of Zoology and Pediatrics and Human Development, Michigan State University, East Lansing, MI 48824.

Smith-Magenis syndrome (SMS) is a multiple congenital anomalies/mental retardation disorder and possible contiguous gene deletion syndrome associated with an interstitial deletion of chromosome 17p11.2. The SMS critical interval has been described, and we have recently completed a BAC/PAC contig across the entire SMS critical interval. The critical interval contains 16 known genes and 12 expressed sequence tags. Haploinsufficiency of one, or many, of the genes in the critical interval may act to manifest the distinct SMS phenotype. We report here the expression pattern of lymphoblastoid cell lines derived from an SMS patient and a parental control using Affymetrix GeneChip<sup>TM</sup> analysis. Lymphoblasts were obtained from fresh blood samples collected from a female SMS patient harboring the common deletion and her phenotypically normal mother. Lymphoblasts were transformed using Epstein-Barr virus. RNA was isolated from the transformed cell lines and reverse transcribed into cDNA, which was then transcribed *in vitro* into biotin labeled cRNA. The cRNA was fragmented and hybridized to Affymetrix GeneChips<sup>TM</sup>. After hybridization, the probe arrays were washed and scanned. The variation in gene expression between the patient cell lines and the parental control indicate which genes in the critical interval may be expressed in a haploinsufficient manner. As well, this study will identify genes outside of the SMS critical interval whose expression may be altered by haploinsufficiency of genes within the SMS region. This is the first study reported to examine the affects of haploinsufficiency toward gene expression utilizing microarray analysis.

**Characterization of a (9,15) translocation in a patient with autism.** S.A. Copeland-Yates<sup>1, 2</sup>, B.R. Dupont<sup>1</sup>, C.D. Skinner<sup>1</sup>, R.C. Michaelis<sup>1</sup>. 1) J.C. Self Research Institute, Greenwood Genetic Center, Greenwood, SC; 2) Department of Biochemistry and Genetics, Clemson University, Clemson, SC.

Autism is a developmental disorder characterized by restricted and repetitive behaviors, deficient social interaction skills, and communication impairments. It is thought to be the most heritable of all pervasive developmental disorders, but to date no disease gene has been associated with autism. Chromosomal translocations can be useful in locating causative genes, as the translocation may disrupt a gene or separate a gene from its regulatory region. In our investigations, we have discovered an autistic patient with a *de novo* balanced translocation involving chromosomes 9 and 15. The translocation breakpoints are located in 9q32 and 15q22. Further studies on this patient using fluorescence *in situ* hybridization (FISH) revealed a bacterial artificial chromosome (BAC) that is disrupted by the breakpoint on chromosome 15. Examination of the sequence of this BAC did not reveal any known genes, but several transcripts are present within the region covered by the BAC. Southern blot analyses using cDNAs corresponding to these transcripts have been performed, but no changes in banding patterns have been found. The location of the breakpoint on chromosome 9 has yet to be defined.

**Male infertility in Kuwait: Molecular and Cytogenetic study.** *F.M. Mohammed<sup>1</sup>, S. AL-FADHLI<sup>1</sup>, O. RUSHDI<sup>2</sup>, S.A. AL-AWADI<sup>3</sup>, K.K. NAGUIB<sup>3</sup>.* 1) Molecular Genetics Laboratory, Faculty of Allied Health, KUWAIT, KUWAIT, KUWAIT; 2) Kuwait Medical Genetics Center, Maternity Hospital, KUWAIT; 3) Family Planning Department, Ministry of Health, KUWAIT.

A case control study of 90 male patients with primary infertility presented by azoospermia/severe oligospermia were studied clinically, cytogenetically and molecularly in order to highlight male infertility in Kuwait. Out of these patients 9 patients (10%) had chromosomal aberration, represented by Klinefelter syndrome/variant (77.8%), XYY syndrome (11.1%) and XX male syndrome (11.1%). Screening of the patient group using the multiplex PCR study revealed detection of microdeletion in one case (1.2%). Six PCR products were deleted in four out of five multiplex reactions and were further confirmed using single PCR reaction. On the other hand, screening for microdeletion in Yq11 using RBM1, RBM2, DAZ and SPGY1 genes revealed undetectable deletion in all patients except the patient who showed microdeletion upon multiplex PCR analysis. He showed deletion of the DAZ and SPGY1 gene specific primer sets. No detectable deletion in the control group was found. The low frequency of microdeletion in the present study may be due to small sample size. Y microdeletion screening is important not only to define the aetiology of spermatogenic failure, but also because it gives precise information for a more appropriate clinical management of the infertile male and his future male child.

**Molecular Cloning of a t(18;21)(p11.1;p1.1) Translocation Associated with Psychosis in one Family. J.M.A.**

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Linkage studies have identified the pericentric region of chromosome 18p as being associated with both bipolar disorder and schizophrenia. We are currently analyzing four cell lines derived from members of a single family. The mother and one son suffered from psychosis whilst two siblings suffered from schizophrenia. Three of the cell lines were cytogenetically identical, carrying a balanced t(18;21)(p11.1;p11.1) translocation as the sole abnormality. The fourth cell line derived from a female schizophrenic with severe symptoms, displayed an unbalanced translocation, having lost the derivative chromosome 21 and gained a normal 21. This resulted in a loss of heterozygosity between 18p11.1-pter. FISH analysis has localized the breakpoint on chromosome 21 to the BAC 2503J9. This clone lies immediately upstream of the published chromosome 21p sequence and contains the TPTE gene, although this gene was not disrupted by the translocation. The breakpoint lies approximately 180 kb telomeric to TPTE. No coding sequences have as yet been detected in the immediate vicinity of the breakpoint on chromosome 21. The breakpoint on chromosome 18 mapped to an unknown distance below IMPA2, a positional and functional candidate gene for psychosis. Although we have yet to identify a gene in this region, the severity of symptoms in the patient with a loss of heterozygosity suggest a role in pathogenesis for a gene on chromosome 18, either at, or proximal to the translocation breakpoint. It is likely that the pericentric and therefore heterochromatic sequences on chromosome 21 exert a regulatory effect in this translocation.

**Implications of chromosome 8p inversions for genetic mapping: neuropsychiatric diseases as an example.** *R.A. Ophoff<sup>1</sup>, S.K. Service<sup>1</sup>, M. Tampilic<sup>1</sup>, M. Eeva<sup>2</sup>, C. Sabatti<sup>2</sup>, A. Palotie<sup>2</sup>, N.B. Freimer<sup>1</sup>*. 1) Center for Neurobehavioral Genetics, Dept of Human Genetics/Psychiatry, UCLA, Los Angeles, CA; 2) Dept of Human Genetics, UCLA, Los Angeles, CA.

It has recently been reported (Giglio et al (2001) *Am J Hum Genet* 68:874-883) that submicroscopic inversions of chromosome 8p of several megabases in length are very common in normal populations. These inversions probably arise from homologous recombination in olfactory receptor gene clusters that flank the inversion, and may have recurred several times in human history. Several independent linkage studies have implicated the region that includes these inversions in susceptibility to neuropsychiatric disorders, including schizophrenia and Tourette syndrome. We recently completed a genome-wide association study of severe bipolar disorder in the isolated population of the Central Valley of Costa Rica, and found the strongest genome-wide evidence for linkage disequilibrium with markers that are contained within or immediately adjacent to these inversions. These common rearrangements could substantially affect statistical analyses of genotyping data from this region and could play a role in disease susceptibility, for example, by altering gene expression in and near the breakpoints.

We have used simulation methods to evaluate the effect of a change of marker order (inversion) on linkage analysis. Additionally, we are defining at the base-pair level, the chromosomal breakpoints, which are heavily loaded with repeat sequences that are present at several other loci in the human genome; this will permit development of simple assays that can be used to identify the frequency of the inversions in affected and unaffected individuals in the studies that have mapped putative neuropsychiatric loci to this region.

**Molecular characterisation of interstitial duplications and triplications involving chromosome 15q11-q13.** *N.S.*

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Chromosome 15 is frequently involved in the formation of structural rearrangements. We report the molecular characterisation of 16 independent interstitial duplications and 3 triplications of the Prader-Willi Angelman critical region (PWACR). Ten duplications were familial, while 5 duplications and all 3 triplications were de novo. The vast majority of PWS and AS deletions include the entire 4 Mb PWACR cassette, and the common deletion breakpoints occur within low copy number repeats flanking the PWACR. All 16 duplications were of an almost uniform size, including two not visible by routine G-banding, and shared the common deletion breakpoints. This implies that some, if not all, duplications and deletions are the reciprocal products of each other. Triplication breakpoints were more variable and extended distally beyond the PWACR, suggesting that structural abnormalities of chromosome 15 involve more than one mechanism. The formation of duplications involved both interchromosomal (n=11) and intrachromosomal (n=5) events. Unlike CMT1A/HNPP rearrangements, duplications and deletions of the PWACR can occur in both male and female meiosis and involve both inter- and intra-chromosomal events. We observed no meiotic instability in the transmission of duplications, although recombination occurred in one family between the normal and duplicated chromosome 15 homologues. The PWACR contains a number of imprinted genes and in all probands except one, the additional copies were maternal in origin. In one family, the duplication was paternal in origin, yet appeared to segregate in a sibship of 3 with an abnormal phenotype, including developmental delay and a behavioural disorder. There was no correlation between rearrangement size, mechanism of origin and clinical severity.

**Deletion of chromosome 2q37.3: report of a case and of candidate genes for autism and osteodystrophy.** *M. Smith, J.R. Escamilla, P. Filipek, M.E. Bocian, C. Modahl, P. Flodman, M.A. Spence.* Dept Pediatrics, Univ California, Irvine, Irvine, CA.

We recently studied a patient who meets criteria for autism disorder and has a deletion of 2q37.3 region on one chromosome. We have defined the extent of the deletion using molecular cytogenetic techniques and 20 non-overlapping BAC clones. We identified new polymorphic markers in the 2q37.3 deletion region. Using these markers we determined that the deletion occurred on the paternally derived chromosome. In the 2q37.3 deletion region we identified four autism candidate genes suggested by the expression of these genes in brain and by neuropathology in autism. Two of the genes that we identified in the 2q37.3 deletion region are abundantly expressed in bone and cartilage. These genes represent candidate genes for the osteodystrophy observed in patients with 2q37.3 deletions. Nine other patients with autism and chromosome 2q37 deletions have been described in the literature. Perhaps certain genetic changes e.g. deletions of specific chromosomal regions, loss of function mutations in critical genes, lead to severe disruption of development and autistic disorder without the presence of additional mutations of autism predisposing genes elsewhere in the genome. A more comprehensive screening of patients with autism is required to determine the frequency of 2q telomeric region deletions. Chromosome 2q telomeric probes for FISH analysis and the polymorphic markers that we identified in the 2q37.3 region serve as screening tools to detect deletions. Also it is possible that in patients without 2q37.3 deletions, examination of polymorphic markers may be informative since specific alleles of these markers may be in linkage disequilibrium with the autism phenotype. In subsequent studies it will be important to search for mutations in candidate genes that map in 2q37.3 in patients with autism who do not have chromosomal abnormalities. Patients with autistic disorder and with chromosomal abnormalities provide an important resource for identification of regions of the genome containing genes important in autism.

**Gene-expression profiles of human small airway epithelial cells treated with low doses of 14- and 16-membered macrolides.** *Y. Yamanaka*<sup>1,3</sup>, *M. Tamari*<sup>2</sup>, *T. Nakahata*<sup>3</sup>, *Y. Nakamura*<sup>1</sup>. 1) Human Genome Center, Inst. of Med. Sci., Univ Tokyo, Tokyo, Japan; 2) SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Tokyo, Japan; 3) Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Although long-term treatment with low doses of 14-membered macrolides is widely applied in management of patients with chronic inflammatory diseases, e.g. diffuse panbronchiolitis, chronic bronchitis, or chronic lung damage in newborns, the physiological mechanisms underlying the action of macrolides in these conditions are unclear. To clarify the pathological basis of these diseases and also to aid in design of novel drugs to treat them, we chose to investigate the molecular target(s) of macrolides. Our experiments involved long-term culture of human small airway epithelial cells (hSAEC) in media containing 14-membered macrolides erythromycin (EM) or clarithromycin (CAM), or a 16-membered macrolide, josamycin (JM), which lacks clinical anti-inflammatory effects. We then analyzed gene-expression profiles in the treated cells using a cDNA microarray consisting of 18,432 genes. We identified nine genes whose expression was significantly altered during 22 days of culture with EM, and seven that were altered by CAM in that time. Four of those genes revealed similar behavior in cells treated with either of the 14-membered macrolides, but not JM. The products of these four genes may be candidates for mediating the ability of 14-membered macrolides to suppress chronic inflammation.



**Association studies of 22 single nucleotide polymorphisms (SNPs) in complement C3, C5, C3aR and C5aR genes for bronchial asthma.** *K. Hasegawa<sup>1</sup>, M. Tamari<sup>1</sup>, X-Q. Mao<sup>2</sup>, T. Enomoto<sup>3</sup>, Y. Dake<sup>3</sup>, S. Doi<sup>4</sup>, H. Fujiwara<sup>4</sup>, A. Miyatake<sup>5</sup>, K. Fujita<sup>5</sup>, Y. Nakamura<sup>6</sup>, T. Shirakawa<sup>1,2</sup>.* 1) Lab. for Functional Analysis, RIKEN SNP Research Center, Tokyo, Japan; 2) Department of Health Promotion & Human Behavior, Kyoto University Graduate School of Public Health, Kyoto, Japan; 3) Department of Otolaryngology, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Japan; 4) Osaka Prefectural Habikino Hospital, Osaka, Japan; 5) Miyatake Asthma Clinic, Osaka, Japan; 6) Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Bronchial asthma (BA), one of the most common of all chronic inflammatory diseases in human populations, is considered to be due to the interaction between environmental and genetic factors. The complement system plays an immunoregulatory role in the interface of innate and acquired immunities. Recent studies provided evidence on the link of C5 and C3a to murine airway hyperresponsiveness, a partial model of human asthma. We searched C3, C5, C3aR and C5aR genes for single nucleotide polymorphisms (SNPs) in the genomic DNAs from BA patients. We have identified 22 SNPs among these genes, and conducted genetic association study in both Japanese (n=384) and British (n=300) populations. A G1896A (Thr612Thr) variant in the C3 gene showed weakly significant association ( $\chi^2=4.29$ ,  $p=0.038$ , Odds ratio=1.56, 95%CI:1.02-2.40) with higher total serum IgE levels (>500IU/ml) in the Japanese childhood BA. Further studies to clarify functional and genetic mechanisms of these genes on the development of asthma may provide a new insight into bronchial asthma.

**Amino-acid substitutions in the IKAP gene product significantly increase risk for bronchial asthma in children.**

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The complex etiology of bronchial asthma (BA), one of the most common inflammatory diseases throughout the world, involves a combination of various genetic and environmental factors. In the course of a project to screen the entire human genome for single nucleotide polymorphisms (SNPs) that might represent useful markers for large-scale association analyses of common diseases and pharmacogenetic traits, we identified six SNPs within the gene encoding I $\kappa$ B-associated protein (IKAP), a regulator of the NF- $\kappa$ B signal pathway. Most of these SNPs were in linkage disequilibrium with each other. We observed a strong allelic association between BA in childhood and two of the SNP sites, T3214A (Cys1072Ser) and C3473T (Pro1158Leu);  $P=0.000004$  for T3214A and  $P=0.0009$  for C3473T. T3214A was also associated with BA in adult patients ( $P=0.000002$ ) but C3473T was not ( $P=0.056$ ). To confirm the above results, we compared estimated frequencies of haplotypes of the six SNPs between BA patients and controls. We found a strong association between BA in childhood and a specific haplotype, TGAAAT, that involved two amino-acid substitutions (819T, 2295G, 2446A, 2490A, 3214A, and 3473T;  $P=0.00004$ , odds ratio=2.94, 95%CI=2.48-3.4). On the other hand haplotype TACGTC, which differed from the TGAAAT haplotype in the last five nucleotides, was inversely correlated with the BA phenotype ( $P=0.002$ , odds ratio=9.83, 95%CI=8.35-11.31). These results indicated that specific variants of the IKAP gene, or a variant in linkage disequilibrium with the TGAAAT haplotype, might be associated with mechanisms responsible for early-onset BA.

**Maternal genetic effects as risk factors for common birth defects: maternal MTRR genotype and spina bifida.**  
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It is generally recognized that the maternal genotype may have an effect on the phenotype of offspring that is in addition to the effect of maternally transmitted alleles. This effect is genetic with respect to the mother but acts as an environmental effect on the offspring. Recent case-control studies of the relationship between genes for folate/cobalamin metabolizing enzymes (MTHFR, MTRR) and spina bifida risk have highlighted the importance of considering both maternal and fetal genotypes as potential risk factors. However, using the case-control approach it is not possible to clearly differentiate between such effects. The two-step transmission disequilibrium test (2sTDT) provides a novel approach for differentiating between maternal and fetal genotypic effects. To assess fetal genotypic effects, transmissions from heterozygous parents to affected offspring are evaluated. To assess maternal genetic effects, the phenotype of interest becomes "having an affected child" and transmissions from heterozygous maternal grandparents of affected individuals, to the mothers of these individuals are evaluated. The 2sTDT was used to evaluate the relationship between variation at the MTRR locus and spina bifida, as part of an ongoing study of genetic and environmental risk factors for this condition. This study was specifically designed to evaluate both maternal and fetal genotypic effects and, hence, includes DNA samples from affected individuals, parents, and maternal grandparents. The MTRR A66G polymorphism, which is a functional variant, was significantly related to having a child with spina bifida. Specifically, heterozygous maternal grandparents of affected individuals transmitted the G allele to the *mothers of offspring with spina bifida* more frequently than expected (12/15,  $P=0.02$ ). In contrast, there was no evidence of excess transmission of the G allele from heterozygous parents to offspring with spina bifida (39/76,  $P=0.82$ ). These results further highlight the importance of assessing both maternal and fetal genotypic effects, and provide the first direct evidence that maternal genetic effects play an important role in the etiology of spina bifida.

**MSX1 Variant is Associated with Cleft Lip/Palate in a South American Population.** *A.R. Vieira<sup>1,2</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) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) ECLAMC at UFRJ, Rio de Janeiro, Brazil; 3) ECLAMC at Fiocruz, Rio de Janeiro, Brazil, and CONICET, Buenos Ires, Argentina; 4) Division of Oral Biology and Dept Human Genetics, Univ 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 and their mothers for molecular analysis. Nonsyndromic oral-facial clefts are common congenital malformations known to occur in approximately 1/1,000 live births in ECLAMC hospitals. The present study used the transmission disequilibrium test (TDT)-based analysis to detect non-Mendelian transmission of one DNA sequence variant in MSX1 to 217 South American children from their respective mothers. The anonymous markers D7S1797 and two others derived from a published sequence for chromosome 1 (HS272L16 Accession # AL023754) were also evaluated and there was no evidence of genome-wide transmission distortion. The results show strong association between nonsyndromic cleft lip with/without cleft palate and MSX1 ( $p=0.002$ ) but not for cleft palate only and MSX1 ( $p=0.33$ ). When cleft lip only cases were analyzed separately, the association with MSX1 was not as strong ( $p=0.025$ ). Also, there was no association between cleft lip with cleft palate and MSX1 ( $p=0.111$ ). When cleft lip with cleft palate and cleft palate only cases were analyzed together, we did not find association with MSX1, although the values were borderline ( $p=0.06$ ). This result supports previous linkage and linkage disequilibrium findings with MSX1 in humans and mice and suggests that MSX1 mutations make a substantial contribution to clefts in South American populations.

**A TDT and candidate gene analysis shows TGFB3 involved in non-syndromic clefting in an Asian population.**  
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Cleft lip and palate is a common congenital structural anomaly with complex etiology involving both genetic and environmental factors. Candidate gene analysis has already suggested the involvement of both MSX1 and TGFB3 in Caucasian populations, and in the case of MSX1, specific etiologic mutations have been identified. TGFB3 is a member of the TGFB family that acts hormonally to control the proliferation and differentiation of multiple cell types. Transgenic knockout mice, in vitro expression and the previous association studies support the involvement of TGFB3 in palatogenesis. To extend the candidate gene approach, we have developed a panel of 500 nuclear triads (father, mother, affected child) with high quality DNA samples drawn from a Philippines non-syndromic cleft lip and palate population. We use 150 of these triads in an initial analysis of common SNP or tandem repeat variants at candidate gene loci and then look for evidence of transmission distortion using the TDT test. These 150 triads have to date been analyzed for the genes GCP2, MTHFR, JAGGED2, MSX1, PAX9, TGFA, and TGFB3. In the case of TGFB3, significant transmission distortion was observed ( $p=0.02$ ) in the initial screening panel of 150 triads. Confirmation of this is now being extended to 350 additional triads. The overall search is also being extended to additional genes whose expression pattern or location suggest a role in clefting. Sequence analysis of TGFB3 exons has identified a new mutation in the promotor region of some NS CL/P families whose analysis is now being extended to these cases, as well. The confirmation of association of TGFB3 with clefting in an Asian population further supports its role in the etiology of non-syndromic clefting and emphasizes the need to carry out additional high-throughput sequence analysis of the coding and regulatory regions of this gene for etiologic mutations.

**Coriell Autism Research Resource: multiplex families and their Hoxa1 polymorphism status.** *R.T. Johnson<sup>1</sup>, L.H. Toji<sup>1</sup>, A. Mars<sup>2</sup>, C.M. Beiswanger<sup>1</sup>, J.C. Leonard<sup>1</sup>, P.K. Bender<sup>1</sup>, J.C. Beck<sup>1</sup>.* 1) Coriell Cell Repositories, Coriell Inst Medical Research, Camden, NJ; 2) Department of Pediatrics, Robert Wood Johnson Medical School, New Brunswick, NJ.

A genetic resource of DNA samples to support the study of autism in families where more than one child is affected or where one child is affected and one demonstrates another significant and related developmental disorder has been built at the Coriell Institute for Medical Research in collaboration with the the University of Medicine and Dentistry Robert Wood Johnson Medical School. An open bank of anonymously collected materials documented by a detailed clinical diagnosis forms the basis of this database of information about the disease. Three criteria were used to assess the autistic phenotype: the ADI (Autism Diagnostic Interview), the ADOS (Autism Diagnostic Observational Schedule-Generic), and the DSM IV Diagnostic Criteria for 299.00 Autistic Disorder. All clinical interviews were conducted face-to-face. For each donor subject tested, a representative Autistic Diagnostic Criteria Score Sheet used to collect data is provided. Currently, there are 28 multiplex families in the Resource including 28 probands and 119 first, second or third degree relatives. 60 individuals have been examined clinically, as described. 44 of these individuals have a diagnosis of autistic disorder by two or more criteria; 37 of these satisfy all three criteria. There are 24 affected sib or first-cousin pairs with a diagnosis of autistic disorder by two or more criteria; 12 of these pairs meet all three criteria. With growing interest in the possible association between HOX genes and autism we have ascertained the frequency of one common polymorphism in exon 1 of Hoxa1 (a single base substitution, A218G), in affected and unaffected families. Our data are in agreement with Ingram et al (*Teratology* 62: 393-405, 2000), and show a similar proportion of subjects in the autistic families with the polymorphic variant. Further information about this resource, including information on ordering, can be found at <http://locus.umdj.edu/autism> or by contact with the Coriell Cell Repositories.

**Mutations in MSX1 are associated with Non-Syndromic Orofacial Clefting.** P. Jezewski<sup>1,4</sup>, A. Vieira<sup>1,2</sup>, R. Schultz<sup>1</sup>, J. Machida<sup>3</sup>, Y. Suzuki<sup>3</sup>, B. Ludwig<sup>1</sup>, S. Daack-Hirsch<sup>1</sup>, S. O'Brien<sup>1</sup>, C. Nishimura<sup>1</sup>, M. Johnson<sup>1</sup>, J.C. Murray<sup>1</sup>. 1) Genetics, Univ Iowa, Iowa City, IA; 2) ECLAMC at UFRJ, Brazil; 3) Aichi-Gakuin Univ, School of Dentistry, Japan; 4) Periodontics, College of Dentistry, Univ Iowa.

Nonsyndromic orofacial clefting is a complex, multifactorial, common birth defect affecting from 1/700 to 1/2500 births. Autosomal dominant, syndromic hypodontia with orofacial clefting is caused by a nonsense mutation in exon 1 of MSX1 while AD selective tooth agenesis is found with an MSX1 change at R196P. Diagnostic resequencing of MSX1 exons and an intronic homology region (IHR) was accomplished in over 1000 cases and controls from Asia, Europe, North and South America, to search for potential mutations in NSOFC. This survey revealed 28 sequence variants, with 13 in the coding regions of the gene. These variants were evaluated as potential etiologic mutations in NSOFC. From the eight amino acid substitutions found, 5 were non-conservative changes. An amino acid sequence homology-based program, SIFT, was used to classify amino acid substitutions as tolerated or deleterious. An R151S substitution was considered intolerant when compared to MSX1 orthologs back to zebrafish. A dizygotic twin with bilateral clefting had G116E while the other twin did not and had unilateral clefting. G116E and V114G were rated intolerant by SIFT when compared to orthologs back to *Xenopus*. E78V, found in several probands from unrelated Filipino families was conserved among mammals. One control individual had a nonconservative amino acid change, G16D, but it was not conserved even in mice. Case and control frequencies were assessed in those variants present at >1% of the population (12/28). Two different point mutations were found in the intronic homology region (IHR) in two Iowa probands but not in any other samples. Cumulatively, these findings point to such variants being etiologic for nonsyndromic orofacial clefting. These results may explain 1-2% of cases of NSOFC and can dramatically change recurrence risks in families from the empiric 3-5% to as high as 50%. Further family, population and functional studies are in progress.

**The HCR susceptibility allele for psoriasis at PSORS1 locus shows altered secondary structure and a possible role in keratinocyte proliferation.** *K. Asumalahti*<sup>1</sup>, *O. Elomaa*<sup>1</sup>, *S. Suomela*<sup>3</sup>, *T. Laitinen*<sup>1</sup>, *R. Ikonen-Vatjus*<sup>5</sup>, *C. Jansen*<sup>4</sup>, *J. Karvonen*<sup>5</sup>, *S-L. Karvonen*<sup>5</sup>, *T. Reunala*<sup>6</sup>, *E. Snellman*<sup>7</sup>, *T. Uurasmaa*<sup>4</sup>, *U. Saarialho-Kere*<sup>3</sup>, *J. Kere*<sup>2</sup>. 1) Dept. of Medical Genetics; 2) Finnish Genome Center, Univ. of Helsinki; 3) Dept. of Dermatology, Helsinki; 4) Turku; 5) Oulu and; 6) Tampere Univ. and; 7) Pajjat-Hame Central Hospitals, Finland.

Psoriasis is a common chronic skin disease characterized by hyperproliferation and loss of differentiation of keratinocytes. We have previously shown that HCR gene at PSORS1 is highly polymorphic and associates strongly with psoriasis. We expanded our family material to become representative of most of Finland. In TDT analysis of 91 Finnish trios we found that four SNPs of the HCR gene form a coding susceptibility allele, HCR\*WWCC, which associates with psoriasis stronger than HLA-Cw\*6 or CDSN\*5. We also verified two new exons in the HCR gene, adding 135 bp 5' of the gene and 26 amino acids to the beginning of the protein. These exons did not contain new polymorphic sites in psoriasis patients. The HCR protein does not have significant homology with previously studied or known proteins. Secondary structure predictions suggest that it contains several segments of coiled-coil structures and contains a Leucine zipper motif. When we compared the structure predictions for the different allelic forms, the psoriasis associated susceptibility allele was predicted to have an altered secondary structure. By immunohistochemical analysis of skin biopsies the HCR protein was seen both in the nuclei and cytoplasm of keratinocytes. In normal and patients' non-lesional skin, staining was detected in basal keratinocytes. However, the staining pattern was different in psoriatic skin where the strongest staining was seen in non-proliferating cells and weak staining in proliferating cells, identified by the expression of the Ki67 proliferation marker. Our results contribute both genetic and functional evidence on the involvement of HCR in psoriasis susceptibility. The HCR gene may have a role in keratinocyte proliferation or differentiation. The altered secondary structure of the susceptibility allele may affect the antigenic properties of the protein.



**Down-regulation of decorin in fibroblasts of patients with classical Ehlers-Danlos syndrome.** *W. Just<sup>1</sup>, I. Hausser<sup>2</sup>, C. Grond-Ginsbach<sup>3</sup>, T. Trautmann<sup>1</sup>, S. Walter<sup>1</sup>.* 1) Dept Human Genetics, Univ Ulm, Ulm, Germany; 2) Dept Dermatology, Univ Heidelberg, Heidelberg, Germany; 3) Dept Neurology, Univ Heidelberg, Heidelberg, Germany.

Ehlers-Danlos syndrome (EDS) is characterized by loose-jointedness and fragile, bruisable skin. EDS I (MIM 130000) is the severe form of classic EDS, and EDS II (MIM 130010) is the milder one. There is evidence that EDS types I and II are allelic, and that mutations in *COL5A1* and *COL5A2* cause the syndrome in about 40%; of the reported investigated cases. In single families, mutations in collagen 1 and the tenascin gene have been detected. Another molecule of the extracellular matrix is decorin. Decorin (*DCN*) is a small leucine-rich proteoglycan (SLRP). *Dcn* null mice show an increased fragility of skin and altered shape and interfibrillar distance of collagen fibrils. Therefore, *DCN* might be a candidate gene for idiopathic fragile skin disorders. We present data about studies in the *DCN* gene of unrelated patients with EDS types I and II, diagnosed according to clinical symptoms and ultrastructural collagen fibril aberrations, who had not been checked for mutations in the *COL5A1/2* genes. We have sequenced all exons with their splice junctions of *DCN* on genomic DNA of 16 EDS I/II individuals. Not a single mutation nor a polymorphism was found. In addition we measured transcript levels in skin fibroblasts of these patients. There is a significant decrease in transcript levels to an average of about 60%; in comparison to healthy controls. Data was obtained from 42 measurements of Northern blots with total or poly-(A)<sup>+</sup>RNA. We suggest that a factor upstream of decorin contributes to the down-regulation of decorin on the mRNA level. In the meanwhile, at least in one of our EDS patients a *COL5A2* mutation was detected. The diminished amounts of processed decorin proteoglycan molecules may be a consequence of other disease-causing mutations and co-influence the precise assembly of collagen fibers, leading to fragile skin and other connective tissue disorders.

This work was supported by the Fritz Thyssen Foundation. Grant: 1999 1071.

**Characterization of a psoriasis locus, PSORS5, on chromosome 3q21.** *L. Samuelsson<sup>1</sup>, D. Hewett<sup>2</sup>, T. Martinsson<sup>1</sup>, J. Riley<sup>2</sup>, I. Purvis<sup>2</sup>, A. Ineroth<sup>3</sup>, J. Wahlstrom<sup>1</sup>.* 1) Clinical Genetics, Sahlgrenska Univ Hosp-East, Gothenburg, Gothenburg, Sweden; 2) UK Discovery Genetics, Glaxo Smith Kline Research & Development, Stevenage, U.K; 3) Dermatology, Sahlgrenska Univ Hosp, Gothenburg, Sweden.

Psoriasis is a chronic inflammatory disease of the skin with both genetic and environmental risk factors. In a genome-wide screen a candidate region on chromosome 3q21 was identified in a Swedish nuclear family set of 134 affected sib-pairs. Linkage was mainly found in families originating from south-west Sweden and the disease locus is likely to be caused by a founder mutation. Likewise, families affected with joint involvement show stronger linkage to the region compared to families without joint involvement. This psoriasis susceptibility locus has been denoted PSORS5. In order to fine-map the PSORS5 locus an SNP map spanning 900-1200 kb was created. 644 individuals from 195 families affected by psoriasis and originating from south-west Sweden were genotyped for a total of 26 SNP markers. The transmission/disequilibrium test (TDT) was used to assess linkage disequilibrium between marker and disease. Five of the 26 SNPs showed significant association ( $p > 0.05$ ). All five markers are located within a 160 kb region. When analysed as a haplotype, alleles of the five markers associated with psoriasis to a p-value of  $< 10^{-7}$ . The 160 kb region was analysed by the Genscan exon-prediction software and a number of putative open-reading frames were identified. Homology searches revealed one previously unidentified gene located in the region. All confirmed and predicted coding sequences have been direct sequenced in 24 psoriatic individuals and 24 healthy control subjects in order to identify potential disease-involved polymorphisms. If there were a significant deviation in allele frequencies between cases and controls, the total family set was genotyped for the polymorphism and results were analysed by the transmission disequilibrium test.

**Insertion/Deletion Polymorphism of the Angiotensin I-Converting Enzyme Gene is Associated with Type 2**

**Diabetes.** *Y. Feng*<sup>1,2</sup>, *T. Niu*<sup>3</sup>, *X. Xu*<sup>3</sup>, *C. Chen*<sup>3</sup>, *Q. Li*<sup>1</sup>, *R. Qian*<sup>4</sup>, *X. Xu*<sup>2,3,5</sup>. 1) Division of Endocrinology, the Third Affiliated Hospital of Beijing University, Beijing, China; 2) Center for Ecogenetics and Reproductive Health, Beijing University, Beijing, China; 3) Prog Population Genetics, Harvard Sch Public Health, Boston, MA; 4) Division of Endocrinology, the First Affiliated Hospital of Beijing University, Beijing, China; 5) The Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

Type 2 diabetes is a complex disorder resulting from the interaction of several genes and environmental factors. To date, major susceptibility genes have not been found for this devastating disease. Angiotensin I-converting enzyme (ACE) is a key enzyme in renin-angiotensin system. Some previous studies suggested that ACE gene insertion/deletion (I/D) polymorphism in intron 16 was associated with vascular complications in type 2 diabetic patients. In an attempt to examine the role of ACE gene I/D polymorphism in type 2 diabetes, we conducted an association study among 132 case-control pairs recruited from northern China. The genotype frequencies for II, ID and DD were 39.8%, 39.8% and 20.3%, respectively in the case group, and 44.8%, 44.8% and 10.4%, respectively in the control group. Comparing DD vs. II/ID genotypes, the DD frequency was found significantly higher in the case group than that in the control group ( $\chi^2=4.77$ ,  $p=0.03$ ). With adjustment of age and gender, the DD genotype was observed to be significantly associated with an increased risk of type 2 diabetes [odds ratio (OR)=2.23, 95% CI: 1.09-4.56]. In conclusion, our data showed that the DD genotype confers increased susceptibility to type 2 diabetes in a population of Chinese ethnicity.

**Gene expression profiling in skeletal muscle of subjects with different glycaemic tolerance.** *C.M. Lindgren<sup>1</sup>, J. Lehar<sup>2</sup>, J. Hirschhorn<sup>2</sup>, A. Vaag<sup>3</sup>, P. Poulssen<sup>3</sup>, K.F. Eriksson<sup>1</sup>, M. Gaassenbeek<sup>2</sup>, C. Ladd<sup>2</sup>, P. Tamayo<sup>2</sup>, M.J. Daly<sup>2</sup>, M. Ridderstråle<sup>1</sup>, H. Beck-Nielsen<sup>3</sup>, E.S. Lander<sup>2</sup>, D. Altshuler<sup>2</sup>, L.C. Groop<sup>1</sup>.* 1) Department of Endocrinology, Institution of Medicine, Lund University, Malmö, Sweden; 2) Whitehead Institute, Center for Genome Research, Massachusetts Institute of Technology, MA, USA; 3) Department of Endocrinology and Internal Medicine, Odense University Hospital.

Type 2 diabetes mellitus is a metabolic disorder caused by impaired insulin action in target tissues (like muscle) and pancreatic beta-cell dysfunction. We hypothesized that variation in the transcriptional process may be associated with differences glucose tolerance and studied variation in gene transcripts in skeletal muscle biopsies between 28 men with normal (age 66±9) and 26 men with abnormal (age 65±9) glucose tolerance, obtained after a euglycemic hyperinsulinemic clamp (Group A). In addition, the acute effect of insulin was evaluated in biopsies taken before and after a euglycemic clamp from 7 monozygotic twin pairs (4 women/10 men) (Group B). Labeled cRNA was analyzed on microarrays including over 6800 genes. Results of group A were analyzed using k-NN statistics and group B by a pair-wise comparison of samples taken before and after insulin infusion. Significance was assessed using permutations although the number of permutations in group B is limited due to the smaller numbers of samples. In the muscle biopsies, we found 209 genes differentially expressed when comparing hyperglycemic men with normoglycemic men (group A),  $p < 0.05$ . In the twins we observed 89 genes which were influenced by insulin (group B),  $p < 0.05$ . In summary, the expression profile in skeletal muscle differs significantly between individuals with different glucose tolerance, which may reflect transcriptional events important in the development and maintenance of abnormal glucose tolerance. The study was, however, not designed to allow conclusions on whether these represent primary changes or develop secondarily to hyperglycemia. A larger group of monozygotic twins may shed light the clues of physiological mechanisms elicited in muscle by insulin stimuli obtained in this study.

**Methylation pattern of a CpG island of ZAC gene in transient and permanent neonatal diabetes mellitus. Y.**

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Depending on their clinical courses, whether a recovery from diabetic condition occurs or not during neonatal period, neonatal diabetes mellitus can be classified into neonatal and permanent types (TNDM and PNDM). In TNDM, paternal duplicated alleles or methylation defect in a maternal allele in a ZAC gene region, a maternally imprinting gene, are responsible for the etiology. However, in a pure form of PNDM, which has no other symptoms except hyperglycemia, molecular defects have not been elucidated so far. In the present study, we analyzed methylation pattern in a CpG island of ZAC gene both in TNDM and PNDM patients. The CpG island extends from a nucleotide number 52460 to 53407 of AL109755 (accession no.). We arbitrary divided the CpG island into two, from 52481 to 52910 and from 52951 to 53260, and named the regions B and A. Then sequencing analysis after bisulfide treatment was performed. In TNDM patients, demethylation pattern of both two regions was observed. However, in four Japanese PNDM patients, the demethylation pattern was observed only in a region A. This methylation pattern could be a molecular defect causing PNDM and might explain the difference of clinical phenotype between TNDM and PNDM.

**SCA8 repeat contractions in the male germline occur before meiosis.** *M.L. Moseley<sup>1</sup>, L. Rasmussen<sup>1</sup>, J. Pryor<sup>2</sup>, K. Roberts<sup>2</sup>, J.W. Day<sup>1</sup>, L.P.W. Ranum<sup>1</sup>.* 1) Institute of Human Genetics, Univ MN, Mpls, MN; 2) Dept Urologic Surgery, Univ MN, Mpls, MN.

Spinocerebellar ataxia type 8 (SCA8) is caused by an untranslated CTG expansion. SCA8 is distinct from other SCAs in that disease penetrance is markedly reduced. Although either parent can transmit the CTG expansion, there is a maternal penetrance bias for transmitting the disease. This bias is consistent with the observation that maternal transmission most frequently results in the expansion of the CTG repeat tract, whereas paternal transmissions almost always result in shorter less penetrant repeat tracts. We evaluated SCA8 instability in sperm samples from individuals with expansions ranging in size from 80 to 800 repeats in blood, and found that the SCA8 repeat tract in sperm underwent contractions in which nearly all of the resulting expanded alleles have repeat lengths of <100 CTGs. The repeat contractions in sperm likely play a role in the reduced penetrance associated with paternal transmissions.

To investigate the origin of these homogeneous and often dramatic contractions in the male germline, we have examined the expansion length in cells at different stages of spermatogenesis. Germ cells from testis tissue of an SCA8 subject were sorted into 2N (spermatogonia), 4N (spermatocytes), and 1N (spermatids) by fluorescence flow cytometry. We found that a 300 repeat allele in blood contracted to a tight window of ~100 repeats in the 2N spermatogonial fraction, a stage of spermatogenesis prior to meiosis. Why the SCA8 expansion contracts during male germ cell mitosis, but is stable in all somatic tissues studied to date remains unclear. One hypothesis is that there may be a growth advantage for spermatogonial stem cells with smaller repeat tracts. If this finding is confirmed in testis samples from other men and other families, it will aid in genetic counseling about the risks of disease transmission in families with an established pathogenic range.

**Genome-wide linkage analysis to presbycusis in the NHLBI Framingham Heart Study.** *A.L. DeStefano<sup>1</sup>, G.A. Gates<sup>2</sup>, N. Heard-Costa<sup>1</sup>, R.H. Myers<sup>1</sup>, C.T. Baldwin<sup>1</sup>.* 1) Boston Univ Sch Medicine, Boston, MA; 2) Virginia Merrill Bloedel Hearing Research Center, Univ Washington, Seattle, WA.

Hearing impairment in the adult population (presbycusis) is estimated to affect 45.9 % of individuals over age 48. Although environmental exposures play a role in age related hearing impairment, studies in both human populations and animal models indicate that there is a genetic component to presbycusis. For this study, standard pure-tone audiograms were obtained on subjects using environments and methods meeting ANSI standards. Pure tone thresholds averaged (PTA) across distinct frequencies (low, medium and high) were used to provide quantitative measures of hearing loss. These measures were also used to define clinical subtypes of which sensory presbycusis, typified by predominately high frequency loss, is the most common. In contrast, strial presbycusis has a relatively flat loss across the low-frequency spectrum with variable degrees of high-frequency loss. While strial presbycusis is less common than sensory presbycusis, it is more heritable ( $h^2 = 0.31 - 0.48$ ). In this study, we conducted a genome wide scan to examine linkage to low and medium PTA in 328 extended pedigrees in the NHLBI's Framingham Heart Study, a community-based longitudinal study in which participants were selected randomly from the population of Framingham, MA. Regression analysis was used to adjust medium and low PTAs for age, age<sup>2</sup> and age<sup>3</sup> and standardized residuals were created. Normalized residuals based on rank were also created and used for analysis. Two-point and multipoint quantitative trait linkage analyses were conducted using the SOLAR package. Six chromosomal locations yielded suggestive evidence of linkage and were located on chromosomes 10, 11, 14 and 18. Several of the regions identified in this study overlap with genes known to cause congenital deafness. Thus, the linkage results suggest that the same genes that cause Mendelian-early onset hearing loss may contribute to age-related hearing loss.

**The Crohn's disease susceptibility gene identified by positional cloning is not associated with rheumatoid arthritis.** *F. Cornelis*<sup>1</sup>, *F. Merlin*<sup>2</sup>, *C. Pierlot*<sup>1</sup>, *S. Moindrault-Cailleau*<sup>1</sup>, *J.P. Hugot*<sup>2</sup>, *S. Lesage*<sup>2</sup> for ECRAF. 1) Laboratoire Europeen de Recherche pour la Polyarthrite Rhumatoide, Universite Paris VII, Evry, France; 2) Fondation Jean Dausset - CEPH, Paris, France.

Crohn's disease and rheumatoid arthritis (RA) are chronic inflammatory diseases of the bowel and joints, respectively. NOD2 was shown to be implicated in Crohn's disease by positional cloning, an insertion deletion polymorphism being the most strongly associated. ECRAF genome scan provided linkage of  $p=0.16$  at that location (D16S411). The aim was to investigate this polymorphism in sporadic and familial RA. Genotyping of the polymorphism was performed on ABI377 for 56 families with 1 RA patient and both parents, as well as in 42 unrelated RA patients from affected sib-pair families. Out of the 56 simplex families, all parents were homozygous for the allele that is not associated with Crohn's disease, but 10 parents who were heterozygotes. From those, the Crohn's allele was transmitted 6 times to the RA offspring. All RA patients from multiplex families were homozygous for the other allele. In conclusion, no evidence was found for a role of that polymorphism in RA susceptibility.



**Fine mapping of the chromosome 16q11-14 susceptibility locus in human SLE sib-pair families.** *P.M. Gaffney<sup>1</sup>, W.A. Ortmann<sup>1</sup>, R.R. Graham<sup>1</sup>, C.D. Langefeld<sup>2</sup>, S.S. Rich<sup>2</sup>, T.W. Behrens<sup>1</sup>*. 1) Department of Medicine, University of Minnesota, Minneapolis, MN; 2) Department of Public Health, Wake Forest University, Winston-Salem, NC.

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease of unknown etiology, with a strong genetic contribution. We have recently reported the results of genome-wide marker screens in SLE sib-pair families, which have identified a region in the centromeric region of the long arm of chromosome 16 that shows strong evidence for linkage in our family collection (LOD 4.86, D16S415). In order to narrow the region for gene identification we have initiated a fine mapping effort in the 16q11-14 region in 193 SLE sib-pair families. Analysis of the first 17 markers on the fine map indicate the best evidence for linkage is found close to marker D16s757 (LOD=2.6), with a 1.5 LOD support interval of approximately 5 cM. Further mapping with both microsatellites and single nucleotide polymorphisms is underway, with a goal of identifying regions exhibiting linkage disequilibrium in our SLE population. The recent identification of the NOD2 gene as the relevant susceptibility gene in this region for Crohns disease, an autoimmune disorder of the gut, has prompted us to examine this gene as a candidate for SLE. Progress in the fine mapping and candidate gene analysis will be presented.

**No support for linkage of chromosome 17<sub>q21-24</sub> to Essential Hypertension in the MRC British Genetics of Hypertension (MRC BRIGHT) Study.** *J. Knight<sup>1</sup>, P. Munroe<sup>1</sup>, W. Lee<sup>2</sup>, N. Brain<sup>2</sup>, J. Pembroke<sup>1</sup>, S. O'Shea<sup>1</sup>, M. Brown<sup>3</sup>, N. Samani<sup>4</sup>, N. Benjamin<sup>1</sup>, M. Farrall<sup>5</sup>, D. Clayton<sup>3</sup>, P. Ratcliffe<sup>5</sup>, J. Webster<sup>6</sup>, M. Lathrop<sup>7</sup>, A. Dominiczak<sup>2</sup>, J. Connell<sup>2</sup>, M. Caulfield<sup>1</sup>.* 1) Barts and the London, UK; 2) Universities of Glasgow; 3) Cambridge; 4) Leicester; 5) Oxford; 6) Aberdeen, UK; 7) CNG, France.

The MRC BRIGHT study is funded to recruit 1500 hypertensive families based upon affected sibling pairs with pre-diagnosis blood pressures in the upper 5% of the blood pressure distribution (145/95mmHg) and onset prior to 60 years of age. Recruitment is now complete and 1700 families have been extensively phenotyped. Recent studies have demonstrated linkage of chromosome 17<sub>q21-24</sub> with human essential hypertension. The BRIGHT resource is ideal for candidate gene/region studies therefore, we assessed whether markers on chromosome 17 previously linked to hypertension are a cause of high blood pressure in this population.

Nine microsatellite markers were selected for analysis in 859 families. All of the markers were amplified using PCR and then genotyped using Applied Biosystems 377 protocols and software. The genotypes were second checked manually and also using a bespoke Microsoft Access data checking tool.

No evidence of linkage was found between the markers studied and essential hypertension in the entire cohort. Maximum likelihood (ML) LOD scores in MAPMAKER/SIBS ranged from 0 to 0.04, in GENEHUNTER the N.P.L. values ranged between -1.6 and 0.19. This data enables us to exclude a gene with a relative risk to a sibling of 1.25 from being present in this chromosomal region. We also analysed increased severity of hypertension as a sub-phenotype. Two groups were selected based on multiple pre-treatment recordings a) 123 families with diastolic readings of 100mmHg or greater and b) 185 families with systolic readings of 160mmHg or greater. Resultant ML LOD scores and N.P.L. values were all 0 or below. Our results suggest if there is a gene(s) on chromosome 17<sub>q21-24</sub> it exerts only a small influence on blood pressure in this population, however it maybe present in other sub-groups of this resource.

**Genetic study of generalized and partial epilepsy - linkage on chromosome 12.** *M.Z. Labuda<sup>1</sup>, S. Bourgoin<sup>1</sup>, A. Gambardella<sup>2</sup>, A. Siren<sup>3</sup>, U. Aguglia<sup>2</sup>, A. Quattrone<sup>2</sup>, E. Andermann<sup>4</sup>, K. Simola<sup>3</sup>, M. Koivikko<sup>3</sup>, M. Pandolfo<sup>1</sup>.* 1) Ctr de Recherche, CHUM, Montreal, Quebec, Canada; 2) Institute of Neurology, School of Medicine, Catanzaro, Italy; 3) Department of Pediatrics, Tampere University Hospital, Finland; 4) Montreal Neurological Institute, Montreal, Quebec, Canada.

We studied seven families with dominantly inherited idiopathic epilepsy. Four families with temporal lobe epilepsy (TLE) had been previously tested for known partial epilepsy loci on chromosomes 20q, 15q, 10q, 8q, 19q 22. Three families with febrile convulsions as the main phenotype were tested for relevant loci on chromosome 2q, 19p, 19q and 11. In all cases, no evidence of linkage was found. Families were then subjected to a genome-wide scan. While three families were previously reported to map on chromosome 5 (ASHG 2000, abstract ), the other two families showed suggestive linkage to chromosome 12. One of these families (5 affected individuals) segregates a TLE phenotype and is from a small community in the Southern Italian region of Calabria, the other one is from Finland and segregates a phenotype that includes febrile convulsion and absence epilepsy (5 affected individuals). We have tested three more small TLE families from the same community in Calabria for linkage to chromosome 12 and found that two are consistent. The cumulative lod score for chromosome 12 markers is 3.6 at  $\theta = 0.0$ . Moreover, another Finnish family previously mapped to chromosome 5 was also consistent with linkage to chromosome 12 with maximum lod score of  $Z=0.72$  at  $\theta=0.1$ ). In addition, and rather surprisingly considering their diverse geographic origin, affected individuals in four families share the same haplotype in a 0.4 Mb interval. However, the frequency of this haplotype on normal chromosomes in the studied populations is 23%. We suggest the possibility of a new epilepsy locus on chromosome 12. In addition, the apparent cosegregation of epilepsy with markers from chromosomes 5 and 12 in a large Finnish family suggests the possibility of digenic contribution to the phenotype.

**Investigation of Chromosome 22 Genes in Rheumatoid Arthritis.** *E. Petit-Teixeira, J. Osorio, G. Bana, S. Moindrault-Cailleau, P. Dieude, C. Pierlot, C. Sardou, S. Lasbleiz, S. Faure, O. Alibert, T. Bardin, B. Prum, F. Cornelis for ECRAF.* Laboratoire de Recherche Europeen pour la Polyarthrite Rhumatoide, Universite Paris VII, Evry, France.

**Background:** Rheumatoid Arthritis (RA) is a multifactorial disease. The chromosome 22 sequence provides a tool to search for RA candidate genes, with the help of RA genome scans performed in Europe (ECRAF), JAPAN and USA (NARAC).

**Objective:** To investigate chromosome 22 genes for RA, using their (putative) function and linkage data.

**Method:** Systematic examination of chromosome 22 genes (known, related and predicted genes), searching for RA candidates based on gene function. Localization of genome scan markers (22/ECRAF, 4/NARAC, 6/JAPAN) within the chromosome 22 sequence along with all genes. Plotting of linkage results according to marker sequence position, defining suggested loci by markers with linkage  $p < 0.05$  and their flanking markers.

**Results:** Out of the 545 genes examined, 18 (3 per cent) were selected as RA candidates, the major candidates being IL17R, MIF and IL2RB. For them, linkage evidence was  $p=0.06$ , 0.5 and 0.5, respectively, as provided by ECRAF closest marker (less than 3cM away), JAPAN and NARAC scan being not contributing (no evidence of linkage with the closest markers). There was one suggested RA locus from linkage data (D22S1160 with  $p = 0.001$ , flanked by D22S274 and D22S1170/ ECRAF), with no gene selected as candidate.

**Conclusion:** The combination of chromosome 22 sequence, with functional information on genes, and linkage data, provided 18 RA candidate genes, with IL17R as number one. Sequence and fine genome scan information, ECRAF genome scan being performed on 1109 markers in total, contributes in prioritizing candidate gene studies in RA. Refinement of linkage data, taking into account the clinical and heterogeneity of the disease, is warranted to take full advantage of the linkage data. The GenHotel program is available to test RA candidates (<http://www.polyarthrite.net>).

**Genomic Screen for POAG susceptibility genes: Follow-up studies and candidate gene evaluation.** *J.L. Wiggs<sup>1</sup>, R.R. Allingham<sup>2</sup>, L.R. Bailey<sup>3</sup>, J. Auguste<sup>1</sup>, E.A. DelBono<sup>1</sup>, B. Broome<sup>2</sup>, F. Lennon Graham<sup>4</sup>, M. Hauser<sup>4</sup>, M.A. Pericak-Vance<sup>4</sup>, J.L. Haines<sup>3</sup>.* 1) Dept Ophthalmology, Mass Eye & Ear Infirmary, Boston, MA; 2) Dept Ophthalmology, Duke University School of Medicine, Durham NC; 3) Program in Human Genetics, Vanderbilt University School of Medicine, Nashville, TN; 4) Center for Human Genetics, Duke University School of Medicine, Durham NC.

We have previously completed an initial genome screen to identify the chromosomal locations of POAG susceptibility genes. This initial study identified 16 chromosomal regions that demonstrated interesting results (lod score >1.0) and/or p-value < 0.05). Additional evidence for POAG loci on chromosomes 2, 4, 14, 15, 17, and 19 was obtained in a second dataset. The purpose of the present study is to further refine these chromosomal regions using additional markers, and including unaffected family members for IBD information, as well as a third group of affected sibling pairs. For this study POAG was defined as age of diagnosis greater than 35, intraocular pressure greater than 22 mm Hg in both eyes, glaucomatous optic nerve damage in both eyes, and visual field loss in at least one eye. Markers that previously demonstrated interesting results as well as near-by markers located 2.5 cM from the original positive markers were analyzed using GENEHUNTER+. Unaffected pedigree members were included in the analysis as unknowns to help identify the parental alleles. The results for the combined dataset continue to show evidence for linkage to all regions, with increased scores for chromosome 15 (from 1.0 to 2.12 for marker D15S165). The remaining regions were basically unchanged. Thus, while chromosomes 2, 4, 14, 17, and 19 remain interesting, the results of this study provide further evidence of a POAG susceptibility gene on chromosome 15. Evaluation of candidate genes within these regions are ongoing. Supported by NIH grant EY10886.

**Genome-Wide Deletion Screen in CHARGE Association.** *S.R. Lalani<sup>1</sup>, C. Bacino<sup>1</sup>, D.W. Stockton<sup>1,4</sup>, L. Molinari<sup>1</sup>, N.L. Glass<sup>2,3</sup>, S.D. Fernbach<sup>1</sup>, J. Towbin<sup>1,3</sup>, W. Craigen<sup>1</sup>, J.W. Belmont<sup>1,3</sup>.* 1) Department of Molecular and Human Genetics; 2) Department of Anesthesia; 3) Department of Pediatrics; 4) Department of Medicine, Baylor College of Medicine, Houston, TX 77030.

CHARGE Association is a common complex of congenital anomalies including ocular coloboma, heart malformation, choanal atresia, retarded growth and development, genitourinary defects, cranial nerve anomalies, and ear abnormalities. Although most evidence points to genetic causation, the etiology of CHARGE is completely unknown. Among possible genetic mechanisms, de novo dominant mutation of an unknown gene with extraordinary pleiotropy or, alternatively, submicroscopic contiguous gene deletion are the most likely. To address the possibility of a deletion as the explanation for at least some cases of CHARGE, we have undertaken genotyping in case/parent trios to detect potential loss of heterozygosity. Extensive phenotypic information has also been collected on each of the 55 affected cases. We have designed a multilevel screening methodology in which an increasingly dense set of markers are used to detect non-mendelian inheritance. For our primary screen, we employed low-density short tandem repeat (STR) markers of 10 cM average distance from ABI Linkage Mapping Set. About 400 markers were used to genotype 10 core trios. Preliminary analysis of the data has shown non-mendelian inheritance pattern in 5 out of 400 markers. Two of these markers, D20S178 and D19S420 have been restudied with fluorescent in situ hybridization (FISH). These experiments showed that the locus was intact on both homologues and preferential allele amplification was the most likely explanation. We are analyzing the other markers using the same methodology. Secondary screening is being initiated with the ABI LMS-HD5 marker set that increases the marker density to an average spacing of 5 cM. We have studied 110 of these 411 markers, with no evidence of loss of heterozygosity. Subsequent screenings at increasing density will be undertaken using additional STRs and single nucleotide polymorphisms (SNPs) to survey markers spaced at 0.1 - 1 Mb intervals.

**Variation At The Nod-2 Locus In Familial And Sporadic Cases Of Crohns Disease In The Ashkenazi Jewish Population.** Z. Zhou, X. Lin, P. Akolkar, B. Gulwani-Akolkar, S. Katz, J. Silver. Department of Medicine, North Shore University Hospital-New York University School of Medicine, Manhasset, NY.

Purpose: Crohns disease (CD), a chronic inflammatory bowel disease, is known to be associated with genetic predisposition. A susceptibility locus, IBD1, has been mapped to the chromosome 16q11-12 region containing the NOD-2 gene. Recent reports indicate that allelic variants in the NOD-2 gene, a member of the CED4/APAF1 superfamily of apoptosis regulators, are associated with CD susceptibility and that homozygosity at this locus for any of three recently defined sequence variants confers a greatly increased risk of CD. These sequence changes include two missense mutations, R675W and G881R, and a frameshift insertion, 980insC. The aim of this study was to determine the frequency of these NOD-2 variants in familial and sporadic CD patients in the Ashkenazi population. Methods: Allele and genotype frequencies of these three variants were determined in 438 CD patients of Jewish descent; 153 patients had a family history of CD and 285 were "sporadic" cases. Variants were detected by PCR using allele-specific primers labeled with fluorescent dye. Results: Although the frequency of the R675W mutation was comparable in familial and sporadic cases (0.053 vs 0.050), the frequency of the G881R and the 980insC mutations was significantly higher in the familial cases than in the sporadic cases (0.137 vs 0.058,  $p=0.000079$ , and 0.091 vs 0.050,  $p=0.020$ ). As a result, the number of CD patients that were homozygous or compound heterozygotes for the NOD-2 mutants was significantly higher in the familial cases than in the sporadic cases (12.4% vs 4.91%,  $p=0.0028$ ). Conclusions: The NOD-2 mutations play a significantly greater role in causing disease in the familial cases than in the sporadic cases. Furthermore, this difference is due to significantly greater frequency of the G881R and 980insC, but not R675W, mutations in the familial cases.

**Approach to defining expression ratio of the SNRPN/B/B' members of the Sm family of spliceosomal proteins.**

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Human small nuclear ribonucleoprotein-associated peptides N, B, and B' (SNRPN or SmN, SNRPB/B' or SmB/B') belong to the Sm family of proteins initially identified by their reactivity with autoimmune sera from patients with systemic lupus erythematosus. Sm proteins bind to small nuclear RNAs U1, U2, U4, and U5, and are thus part of the spliceosome, but the functions of the individual polypeptides have yet to be completely determined. The SmN locus is imprinted and has been implicated in Prader-Willie syndrome (PWS), a neurogenetic disorder caused by the loss of gene(s) from paternal 15q11-q13. Expression studies at the mRNA and protein levels for humans and a mouse model of PWS have suggested a tissue-specific pattern for SmN and its murine homolog. Furthermore, a compensatory feedback loop has been proposed to regulate levels of SmB/B' and SmN. However, a clear picture of expression patterns of all three human proteins at the RNA level has yet to be achieved. The possible tissue-specific expression patterns of SmN and its murine homolog have lead to suggestions that they play a role in tissue-specific alternative splicing, possibly involving recognition of cryptic splice sites. The goal of this study is to determine at the mRNA level the expression ratio of the SmB, SmB', and SmN proteins in different tissues. Total RNA was extracted from frozen human tissue samples by the single-step guanidinium acid-phenol method. After RT-nested PCRs, PCR products were then separated in an 8% polyacrylamide gel and visualized by silver staining. Initial results have indicated that SmB is ubiquitous, as expected. The SmB' system is currently being optimized. For SmN, the expected size product was detectable in all tissues examined, with multiple bands present in some tissues.



**Linkage and association analysis of chromosome 3 in autistic disorder.** *M.A. Pericak-Vance<sup>1</sup>, Y. Shao<sup>1</sup>, K. Raiford<sup>1</sup>, C.M. Wolpert<sup>1</sup>, H.H. Wright<sup>2</sup>, R.K. Abramson<sup>2</sup>, G.R. DeLong<sup>1</sup>, M.L. Cuccaro<sup>2</sup>, J.R. Gilbert<sup>1</sup>.* 1) DukeCtr Human Genetics, Duke Univ Medical Ctr, Durham, NC; 2) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Genomic screen analysis of 99 multiplex Autistic Disorder (AutD) revealed evidence for linkage on chromosome 3 with D3S3680 (36.1 cM) resulting in a peak two point MLS=2.02. Subsequently we genotyped an additional 90 multiplex AutD families from the Autism Genetic Resource Exchange (AGRE) for markers in this region. With the new data the two point MLS score for D3S3680 increased to 3.16. Several other markers in the region also gave support for linkage in the expanded dataset including D3S4545 (26.3 cM), D3S3589 (32.3 cM) and D3S1259 (36.7 cM) with MLS=2.32,1.95 and 1.16, respectively. The peak multipoint MLS was at D3S1259 (MLS=2.66). The oxytocin receptor gene (OXTR) is located on chromosome 3p25-p26 near our peak region. Animal models have shown that OXTR is expressed in brain as well as uterus and mammary gland and has an effect on neural development. OXTR has also been shown to induce stereotypic behaviors in mice. To evaluate the OXTR as a potential AutD candidate gene we genotyped several SNPs in OXTR and tested for association using 2 different family-based association methods, the pedigree disequilibrium test (PDT) and TRANSMIT (TMT) in the combined AutD dataset. We found no evidence for association with either method in any of the OXTR SNPs. These data suggest that the OXTR gene is not a major contributor to risk in AutD, however, the region on chromosome 3 potentially harbors an AutD susceptibility gene.

**Autism DNA methylation profiling using CpG microarrays.** *C.H. Wang<sup>1,2,4</sup>, Y. Liu<sup>2</sup>, P.S. Yan<sup>3</sup>, T. Huang<sup>3</sup>, J.H. Miles<sup>4</sup>, R.E. Hillman<sup>4</sup>.* 1) Dept of Psychiatry and Neurology, Univ Missouri, Columbia, MO; 2) Dept of Biochemistry, Univ Missouri, Columbia, MO; 3) Dept of Pathology and Anatomical Sciences, Univ Missouri, Columbia, MO; 4) Dept of Child Health, Univ Missouri, Columbia, MO.

Autism is a complex neurodevelopmental disorder with a multigenic etiology. The wide spectrum of autism phenotypes may be the result of aberrant expression of many susceptibility genes. DNA methylation is a recognized regulatory mechanism of gene expression. Abnormal genomic methylation may underlie the pathogenesis of autism. In order to study the epigenetic influence of DNA methylation on autism, we applied a differential methylation hybridization (DMH) method to isolate the CpG islands that are abnormally methylated in autism. CpG microarrays were constructed from a CGI library enriched for CpG islands. Individual CGI clones were selected against repetitive sequences and amplified by colony PCR. A total of 768 CGI clones, each containing at least one methylation sensitive BstUI site, were manually dotted, in duplicate, onto a nylon membrane. Forty DNA samples were selected, 20 each from autism and control groups. Total genomic DNA was digested with MseI, subtracted from repetitive sequences, then digested with BstUI. The methylated fragments (undigested by BstUI) were PCR amplified, radiolabeled, and hybridized to the CpG microarray membranes. Each membrane was used only once. The radiodensities of each hybridized CGI clone were captured by a phosphoimager and analyzed by an ImageQuant software. We have identified at least three interesting clones. One clone is hypomethylated in the autism genome and emits a mean density of 1.1053 among autism samples, as opposed to 2.4375 among the controls. Two other clones are hypermethylated in the autism genome. One emits a mean density of 8.8947 in autism and 6.3125 in control samples. The other emits a mean density of 5.8947 in autism and 4.5000 in control samples. These data suggest that aberrant DNA methylation may be observed in the CpG islands within the autism genome. We are currently isolating these CGI clones in order to relate their sequences to functional genes.

**Candidate gene analysis of cleft lip/palate with hypodontia outside the cleft.** *R.L. Slayton<sup>1</sup>, L. Williams<sup>1</sup>, J.C. Murray<sup>1</sup>, J.J. Wheeler<sup>1</sup>, A.C. Lidral<sup>2</sup>, C. Nishimura<sup>1</sup>.* 1) The University of Iowa, Iowa City, IA; 2) The Ohio State University, Columbus, OH.

The etiology of orofacial clefting is complex and involves both genetic and environmental factors. Dental anomalies associated with cleft lip and/or palate (CL/P) include variations in number, size and position of developing teeth. Evidence for a similar genetic etiology for both orofacial clefting and hypodontia comes from a variety of sources, including knock-out mouse models and single gene disorders where both clefting and hypodontia are observed. The purpose of this pilot study was to determine if the candidate genes previously studied in subjects with CL/P are associated with hypodontia outside the region of the cleft. Subjects were ascertained as a population-based case-control study within the University of Iowa Craniofacial Anomalies Research Center (CARC). A subset of the CARC subjects were selected based on the availability of both dental records and genotyping information. One hundred twenty subjects met both criteria. A dental chart review was performed to verify the type of orofacial clefting and to identify the type and location of dental anomalies present (including missing teeth, supernumerary teeth and microdontia). Polymorphic markers in candidate genes were screened using PCR/SSCP. Candidate genes included MSX1, TGFB3, TGFA and PAX9. Chi-square analysis was used to evaluate the frequency of alleles for cases and controls for each SNP and the CLUMP program was used to analyze di-nucleotide repeat polymorphisms. The overall prevalence of hypodontia in this sample was 46.7% with 28.3% of subjects having missing teeth outside of the cleft. Dental anomalies were more frequently associated with CL/P than with CPO ( $p < 0.01$ ). In addition, hypodontia outside the cleft region was more likely to occur in subjects with CLP than in CL or CPO ( $p < 0.01$ ). Analysis of candidate genes found no significant association between the subjects with hypodontia outside the cleft region when compared to either non-cleft controls or controls with clefting but no hypodontia outside the cleft region.

**The *hairless* gene in androgenetic alopecia: Results of a systematic mutation screening and a family-based association approach.** A.M. Hillmer<sup>1</sup>, R. Kruse<sup>2</sup>, F. Macciardi<sup>3</sup>, U. Heyn<sup>1</sup>, R.C. Betz<sup>1</sup>, T. Ruzicka<sup>2</sup>, P. Propping<sup>1</sup>, M.M. Noethen<sup>1,4</sup>, S. Cichon<sup>1,4</sup>. 1) Institute of Human Genetics, University of Bonn, Bonn, Germany; 2) Department of Dermatology, University of Duesseldorf, Duesseldorf, Germany; 3) Unit of Biostatistics and Genetic Epidemiology, University of Toronto, Toronto, Canada; 4) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium.

Genetic disposition and androgen dependence are important characteristics of the common patterned loss of scalp hair known as androgenetic alopecia (AGA). The genetic factors contributing to AGA are currently unknown. Recently, the human *hairless* gene (*HR*) has been cloned and mutations have been reported in families with autosomal recessive universal congenital alopecia and papular atrichia. The main feature of these disorders is persistent complete absence of hair at or shortly after birth. This suggests that *HR* is essential and specific for the development of hair. Therefore, *HR* can be considered a candidate for involvement in AGA. To test this hypothesis, we systematically screened the *HR* gene for genetic variability by means of single strand conformation analysis in 46 unrelated men with AGA. We detected 15 single nucleotide substitutions including eight missense mutations (205C®T, 1010G®A, 1190G®A, 1577T®C, 1727C®T, 1772A®G, 1859G®A, 3064A®G), four silent mutations (1305T®C, 2370T®C, 3234C®G, 3339C®A), and three mutations in exon-flanking intronic sequences (2611-68C®T, 2611-52T®C, 3379-29G®T). To test for an involvement of the *HR* gene in the development of AGA, seven common variants were genotyped in 61 families, yielding a total sample of 93 parent-offspring trios. The results were analyzed with the transmission/disequilibrium test (TDT). A significant association was found between AGA and variants 3379-29G/T (p=0.024) and 2611-68C/T (p=0.047), respectively. This result suggests an involvement of the *HR* gene in the development of AGA. Definite conclusions, however, require replication of the finding in independent samples.

**Alleles of the putative taste receptor gene TAS1R3 may influence the pleasantness of sucrose and aspartame in human subjects.** *D.R. Reed<sup>1</sup>, X. Li<sup>1</sup>, Z. Chen<sup>1</sup>, A.A. Bachmanov<sup>1</sup>, G.K. Beauchamp<sup>1</sup>, M.G. Tordoff<sup>1</sup>, M. Max<sup>2</sup>, R. Margolskee<sup>2</sup>, L.M. Bartoshuk<sup>3</sup>, V. Duffy<sup>3,4</sup>.* 1) Monell Chemical Senses Center, Philadelphia, PA; 2) Mount Sinai School of Medicine, New York, NY; 3) Yale University, New Haven, CT; 4) University of Connecticut, Storrs, CT.

The *Tas1r3* gene is a candidate for the saccharin preference locus (*Sac*), a locus that is allelic in mice, and that influences the intake and preference for substances humans report as sweet. For instance, mice with the *Sac<sup>d</sup>* allele drink equal amounts of water and 1.6 mM saccharin when offered both choices, whereas those with the *Sac<sup>b</sup>* allele drink 90% of their fluid as saccharin. A missense mutation (I60T) in the mouse is currently the most likely mutation to account for the phenotypic differences. The human ortholog of *Tas1r3* exists on chromosome 1p36. To determine whether allelic variants of this gene exist in humans, and are associated with differences in human behavior toward sucrose and aspartame, the TAS1R3 genes of 30 unrelated human subjects were sequenced. Three sequence variants that resulted in a predicted protein change were found. Twenty-three additional subjects were genotyped and phenotyped. In following up on these polymorphisms, six heterozygotes and forty-seven "wt" homozygotes subjects were compared for their ratings of intensity and pleasantness. Heterozygous subjects reported that 5% ( $p=0.13$ ), 10% ( $p=0.021$ ), and 20% ( $p=0.0011$ ) sucrose was more pleasant than subjects with the homozygous genotype. Subjects with the heterozygous genotype also rated aspartame as more pleasant than those with the homozygous genotype ( $p=0.05$ ). Intensity ratings did not differ between genotypes. The mutation present in the heterozygotes is predicted to act as "gain of function" mutation to alter receptor activity. Further work is needed to determine whether this effect of genotype is specific to sweet taste and extrapolates to other human populations.

**A genome-wide association study of psoriasis vulgaris using polymorphic microsatellite markers and microarray**

**technology.** *G. Tamiya<sup>1</sup>, A. Oka<sup>1</sup>, K. Okamoto<sup>1,2</sup>, T. Endo<sup>3</sup>, S. Makino<sup>1</sup>, H. Hayashi<sup>1</sup>, M. Iizuka<sup>4</sup>, E. Tokubo<sup>1</sup>, R. Sato<sup>1,2</sup>, A. Takaki<sup>1</sup>, Y. Sakurai<sup>1</sup>, M. Ota<sup>5</sup>, Y. Nagatsuka<sup>1</sup>, T. Imanishi<sup>3</sup>, T. Gojobori<sup>3</sup>, A. Ozawa<sup>4</sup>, H. Inoko<sup>1</sup>.* 1) Dept Molecular Lifescience II, Sch Medicine, Tokai Univ, Isehara, Japan; 2) Chugai Pharmaceutical Co., Ltd, Gotemba, Japan; 3) National Institute of Genetics, Mishima, Japan; 4) Dept Dermatology, Sch Medicine, Tokai Univ, Isehara, Japan; 5) Shinshu Univ, Sch Medicine, Matsumoto, Japan.

The aim of this study is to exclusively identify genes related to psoriasis vulgaris by genome-wide association analysis using polymorphic microsatellite markers. Our previous data suggested that microsatellite markers showed a linkage disequilibrium with disease-related alleles spanning from 100 to 200 kilobases (kb). For the purpose of the genome-wide association studies, we identified and designed primers for microsatellite sequences from the human genome draft sequence, resulting in the assignment of 30,000 polymorphic microsatellite markers which are separated by 100 kb intervals. From this genotype study we have specifically identified the polymorphic markers which we plan to submit to the public genomic repository. These identified polymorphic markers within the Japanese population include previously published markers such as the Genethon markers as well as our newly identified markers. We have applied the polymorphic markers to conduct a genome-wide association study of psoriasis vulgaris-susceptibility genes. We report here our findings to date of different susceptibility locations within the genome including confirmation of our previous findings that identified a psoriasis-susceptibility locus near the HLA-C gene within the MHC region of chromosome 6. In addition, we report our preliminary findings on the comparison of gene expression in human affected and normal skin using the expression microarray system. We propose that the information gathered from these highly upregulated/downregulated and normally expressed genes in diseased skin using the DNA microarray technology will complement our genome-wide association studies. We believe this combined approach will accelerate identification of disease-related genes.

**Progress toward characterization and gene analysis of distal translocation breakpoint at 7q31.2 in Autistic disorder.** *P.-T. Xu, P. McCoy, M.M. Menold, J.M. Vance, J.R. Gilbert, M.A. Pericak-Vance.* Department of Medicine, Duke Univ Medical Ctr, Durham, NC.

Autistic disorder (AutD) is a severe neurodevelopmental disorder characterized by impairments in reciprocal social interaction and communication, and restricted and stereotyped patterns of interests and activities, with an onset before three years of age. Multiple genome screens has indicated an AutD susceptibility locus on chromosome 7q. We have identified an AutD family in which three sibs inherited from their mother a paracentric inversion in the chromosome 7 (Ch 7) candidate region [inv(7)(q22-q31.2)]. To further identify the location of distal breakpoint at Ch 7q31 region, we utilized available Ch 7 genomic sequence of Ch 7 BACs and/or PACs to refine mapping and sequencing data in the inversion region and overlap a region of 2,500,000 base pair of genomic sequence. Based on the genomic sequence we have developed 9 probes for pulse field gel analyses (PFGE). Patient and control genomic DNAs from inversion family and other AutD families were analyzed using multiple rare-cut restriction enzymes. Preliminary results demonstrate that the distal breakpoint may localize in a PFGE map and gap area estimated about 100 kb (approximately 109,000,000 ~110,000,000). The patterns and status of gene expression and transcription in inversion patients are presented.

**Identification of candidate genes for Parkinson's Disease (PD) by profiling gene expression in the substantia nigra.**

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Parkinson's Disease (PD) is a heritable neurological disorder characterized by pathological degeneration of the substantia nigra, most notably loss of dopaminergic neurons. We hypothesize that this degeneration is the result of an altered pattern of gene expression in this tissue. Genes whose expression in the substantia nigra are significantly up- or down regulated in affected individuals therefore constitute good candidate susceptibility genes for this disorder. Additional candidate genes will be identified as those genes that are expressed in the normal substantia nigra at higher levels than in other regions of normal human brain. Candidate genes will then be analyzed by family based association studies using nearby single nucleotide polymorphisms (SNPs). We are prioritizing analysis of candidate genes that display association and also map to regions linked to PD in our recent genome scan. Serial Analysis of Gene Expression (SAGE) and Affymetrix microarray analysis are being used to profile gene expression in substantia nigra tissue collected from PD patients and age-matched controls. SAGE is a powerful technique that allows quantitative determination of all transcripts present in a given tissue by cloning and sequencing large numbers of unique 13-base pair tags associated with each gene. We present here a detailed profile of genes expressed in the normal control substantia nigra, as compared with other regions of the brain. The 10 most abundantly expressed genes in the control substantia nigra are KIAA0493, gelsolin, osteonectin, sperm associated antigen 7, cystatin C, KIAA1378, leucine rich repeat (in FLII) interacting protein 1, KIAA0729, carboxypeptidase M, and chondroitin 4-O-sulfotransferase 2.



**Multiplex analysis of 65 polymorphisms in coronary artery disease patients.** *C. Stranieri<sup>1</sup>, E. Trabetti<sup>1</sup>, G. Malerba<sup>1</sup>, M. Grow<sup>2</sup>, A. Silbergleit<sup>2</sup>, C. Carlson<sup>2</sup>, D. Girelli<sup>3</sup>, O. Olivieri<sup>3</sup>, R. Corrocher<sup>3</sup>, P.F. Pignatti<sup>1</sup>, H. Erlich<sup>2</sup>, S. Cheng<sup>2</sup>.* 1) Sect Biol & Genetics, Mother-Child, Biol & Genetics, Verona, Italy; 2) Dpt.of Hum. Genetics, Roche Molecular System, Inc., Alameda, CA, USA; 3) Dpt. of Exp. and Clin. Med., Chair of Internal Medicine, Verona, Italy.

Several genes may contribute to the susceptibility to cardiovascular disease, together with environmental factors. The goal of the present study was to test a prototype multiplex PCR- and immobilized probe-based assay to genotype up to 65 variable sites in 36 candidate genes, and possibly correlate genotypes to the risk of coronary atherosclerosis. The polymorphisms are located in genes involved in lipid (apo(a), ApoAIV, ApoB, ApoCIII, ApoE, b3AR, CETP, LDLR, LIPH, LPL, PON1, PON2, PPAR $\gamma$ ) or homocysteine metabolism (CBS, MTHFR), coagulation (FII, FV, FVII, FBG, PAI-1 GpIa, GpIIIa), leukocyte adhesion (ELAM, ICAM), blood pressure regulation (ACE, ATIIR, AGT, STMY1, a adducin, ANP, ADRb2, ENaCa, GNB3), and inflammation (NOS3, TNFa, TNFb). Two groups of subjects from the same population presenting for cardiovascular surgery were selected: 756 with angiographically documented coronary artery disease (CAD+), and 320 with angiographically documented normal coronary arteries (CAD-, controls). Allele and genotype frequencies were estimated in each group. Significant differences were observed in genotype distribution between patients and controls for two ApoCIII gene promoter polymorphisms -455T/C and -641C/A (P=0.002), and the ADRB2 gene Gln27Glu polymorphism (P=0.03). The two ApoCIII gene polymorphisms were in linkage disequilibrium (D'=0.98). The -455C/C (-641A/A) homozygotes were 1.9 times more frequent in cases relative to controls (OR 2.11, 1.38-3.25, P=0.0003). ApoCIII -455 T/C (-641C/A) genotyping, in conjunction with the determination of other genetic and environmental risk factors, may help in estimating CAD susceptibility in at risk individuals.

**Insulin degrading enzyme is not associated with late-onset Alzheimer's disease.** R. Abraham<sup>1</sup>, M. Hamshere<sup>1</sup>, A. Myers<sup>2</sup>, J. Kwon<sup>2</sup>, G. Spurlock<sup>1</sup>, H. Thomas<sup>1</sup>, D. Turic<sup>1</sup>, H. Marshall<sup>2</sup>, F. Wavrant De-Vrieze<sup>3</sup>, D. Compton<sup>3</sup>, B. Hoogendoorn<sup>1</sup>, D. Liolitsa<sup>4</sup>, S. Lovestone<sup>4</sup>, J. Hardy<sup>3</sup>, A. Goate<sup>2</sup>, M. O'Donovan<sup>1</sup>, J. Williams<sup>1</sup>, M.J. Owen<sup>1</sup>, L. Jones<sup>1</sup>. 1) Department of Psychological Medicine, University of Wales College of Medicine, Cardiff, UK; 2) Department of Psychiatry, Washington University School of Medicine, 660 S Euclid, St Louis, MO63110, USA; 3) Laboratory for Neurogenetics, Department of Neuroscience, Mayo Clinic Jacksonville, FL32224, USA; 4) Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF, UK.

Insulin degrading enzyme (IDE; insulysin; EC 3.4.24.56) is a 110kDa neutral metallopeptidase which can degrade a number of peptides including b-amyloid. It is located on chromosome 10 close to a region of linkage for late-onset Alzheimer's disease (AD) and thus is a functional and positional candidate for this disorder. We analysed all the coding exons, untranslated regions and 1000bp of 5' flanking sequence of *IDE* using DHPLC and sequencing to detect sequence variants. We detected eight single nucleotide polymorphisms (SNPs), three in the 5' flanking sequence and five in the coding sequence, of which three were found at <5% frequency. None changed the amino acid sequence. We tested the five SNPs with allele frequencies of >5% in two case-control samples, 133 caucasian late-onset AD cases and 135 controls collected in the UK and 95 caucasian late-onset AD cases and 117 controls collected in the USA. No association of any SNP with late-onset AD was detected. Two of the SNPs were analysed in 86 late-onset AD cases and 94 controls, caucasians, and again no association with late-onset AD was found. Analysis of marker D10S583, which maps 36kb upstream of *IDE* and was previously reported to be associated with AD failed to show association in the UK sample. Substantial LD was detected between the SNPs across the *IDE* gene. We conclude that *IDE* does not make a substantial contribution to late-onset AD, and cannot therefore account for the linkage between late-onset AD and 10q.

**COMT Val158Met and cognitive executive function.** *D. Goldman*<sup>1</sup>, *A. Malhotra*<sup>3</sup>, *M. Egan*<sup>2</sup>, *B. Kolachana*<sup>2</sup>, *D. Warden*<sup>4</sup>, *R.H. Lipsky*<sup>1</sup>, *A.M. Salazar*<sup>4</sup>, *K. Xu*<sup>1</sup>, *T. Goldberg*<sup>2</sup>, *D. Weinberger*<sup>2</sup>. 1) Laboratory of Neurogenetics, NIAAA, Rockville, MD; 2) Clinical Brain Disorders Branch, NIMH; 3) Hillside Hospital, Long Island, NY; 4) Walter Reed Army Hospital, Washington, DC.

Impaired prefrontal cognitive function is a hallmark of schizophrenia, behavioral disinhibition syndrome following head injury, and various psychiatric diseases involving impaired impulse control, for example alcoholism. Augmentation of dopamine in frontal cortex improves performance on tasks that access function of this brain region and simultaneously increases metabolic efficiency, probably through an action on dopamine D1 receptors [P. Goldman-Rakic. An abundant functional polymorphism [candidate allele] of the D1 receptor is not known. However, Val158Met, a common [rare allele=0.42] polymorphism, exerts a four-fold effect on activity of catechol-O-methyl transferase [COMT], which catabolizes dopamine and other catecholamines. We hypothesized that Val158, associated with more rapid dopamine metabolism, would lead to poorer frontal cognitive performance, reduced cortical efficiency, and increased risk of schizophrenia. WCST perseverative errors were predicted by Val158Met genotype in three datasets: Jackson Foundation Head Injury [N=188], Hillside Hospital controls[N=73] and NIMH [N=449]; the NIMH dataset included 175 schizophrenia patients, 219 unaffected siblings and 55 controls. Linkage to schizophrenia was evaluated by the transmission disequilibrium test [TDT]. Also, in part of the NIMH sample, brain metabolic activity was measured by BOLD-MRI during a working memory task. Significant association between Val158 and WCST perseverative errors was seen in all three datasets, despite differences in baseline WCST performance in the several clinical groups. Significant case/control and TDT associations between Val158 and schizophrenia were detected. Finally, Val158 genotypes were associated with cortical inefficiency in the dorsolateral frontal cortex and anterior cingulate regions. These results, obtained in several datasets with extended psychiatric phenotypes, suggest a role for Val158Met in executive cognition, frontal cortical metabolism, and schizophrenia vulnerability.

**Are CFTR gene mutations involved in a subgroup of sarcoidosis patients?** *C. Bombieri<sup>1</sup>, F. Belpinati<sup>1</sup>, M. Zorzetto<sup>2</sup>, I. Ferrarotti<sup>2</sup>, M. Luisetti<sup>2</sup>, P.F. Pignatti<sup>1</sup>*. 1) Section of Biology & Genetics, DMIBG, University of Verona, Verona, Italy; 2) Lab. of Biochemistry & Genetics, Inst. Respiratory Diseases, IRCCS S.Matteo, Univ. of Pavia, Pavia, Italy.

Sarcoidosis is a multifactorial, multisystem immune-mediated granulomatous disorder for which ethnic variation in incidence, and familial clustering, suggest the presence of a genetic component. In a previous investigation (34 patients) we had evidence of an excess of CFTR gene mutations in sarcoidosis patients. A trend towards increased chest X-ray progression was observed in patients with CFTR gene mutations compared with patients without mutations (*Eur. J. Hum. Genet.* 2000; 8:717). In order to increase sample numerosity, 19 new patients with sarcoidosis were enrolled in this study. A DGGE analysis was performed on all exons and intronic flanking regions of the CFTR gene. At the writing of this abstract the analysis is completed for all the gene except one exon. We found one CFTR gene mutation in 3/19 patients (R31C, D513G, G576A-R668C). This is not statistically different from controls. Combining these results with the previous findings, a total of 16/53 (30%) sarcoidosis patients have at least one CFTR mutations, an increase which is not statistically different from controls (10/50) taken from the general population (*Hum. Genet.* 2000; 106:172). The presence of CFTR gene mutations will be correlated with disease progression and with mutations in other genes possibly involved in the disease susceptibility.

**Complex multigenic inheritance influences the development of severe CF liver disease.** *K.J. Friedman<sup>1</sup>, P.R. Durie<sup>2</sup>, L.M. Silverman<sup>1</sup>, M.R. Knowles<sup>1</sup> and the Cystic Fibrosis Liver Disease Consortium.* 1) Depts Pathology & Lab Medicine, and Medicine, Univ North Carolina, Chapel Hill, NC; 2) Dept Pediatrics, Univ Toronto, Ontario, Canada.

Severe liver disease afflicts 2-7% of patients with CF. The risk for CF liver disease (CFLD) is likely influenced by genetic factors. Previously we reported an increased frequency of protease inhibitor (PI) gene mutations, Z & S, among CFLD patients with portal hypertension & cirrhosis. This relationship does not exclude mutations or sequence variants at other loci from contributing to severe CFLD. The mannose binding lectin (MBL2) gene is an adverse modifier of CF lung disease and has been explored in regard to CFLD. We have genotyped 53 CFLD patients for promoter variant -221 X/Y and structural mutations B, C, & D, each associated with reduced MBL2 serum levels. None of these variants is significantly elevated among CFLD patients as a group compared to normal controls. However, CFLD patients (n=42) without PI mutations are more likely to carry two structural MBL2 variants than controls (p=.057, OR 3.7, 95%CI 1.02-13.1), suggesting that mutations in the PI and MBL2 genes are independent risk factors for CFLD. The TGF $\beta$  gene was also evaluated in CFLD patients, as this cytokine mediates the hepatic fibrogenesis initiated by cholangiocytes compromised by CFTR mutations. A high-expression variant of the TGF $\beta$  promoter (T @ -509) might exacerbate the fibrogenic stimuli inherent to CFLD. Although there is no increased frequency of genotypes bearing TGF $\beta$  -509 T among CFLD patients as a whole, 10 of 11 (91%) CFLD patients who also carry a Z or S allele in the PI gene carry one or two -509 T alleles, vs. 55% among normals (p=.026, OR 8.3, 95%CI 1.1-65.6). Furthermore, 3 of these 11 patients (27%) are TT homozygotes compared to 7.5% among normals (p=.05, OR 4.7, 95%CI 1.2-18.7). We also note that 13 CF DF508 homozygous controls without liver disease who also carried a Z or S allele were less likely to carry one or two TGF $\beta$  -509 T alleles than the 11 CFLD patients above (p=.03, OR 11.7, 95%CI 1.14-119.6). Taken together, high expression of TGF $\beta$  may potentiate the adverse impact of PI gene mutations on CFLD.

**Crohn's disease: analysis of the IBD1 frameshift mutation versus linkage evidence.** *J.A. Cavanaugh<sup>1</sup>, K.E. Adams<sup>1</sup>, E.J. Quak<sup>1</sup>, N.R. Beck<sup>1</sup>, P. Pavli<sup>1</sup>, S.R. Wilson<sup>2</sup>, N.J. O'Callaghan<sup>3</sup>, W. Uylaki<sup>4</sup>, J. Eaden<sup>4</sup>, I. Roberts-Thomson<sup>4</sup>, D.F. Callen<sup>3</sup>.* 1) Gastroenterology Unit, Canberra Hosp, Canberra, Australia; 2) Centre for Mathematics and its Applications, Australian National University, Canberra, Australia; 3) Department of Cytogenetics and Molecular Genetics, Women's & Children's Hospital, Adelaide, Australia; 4) Department of Gastroenterology, Queen Elizabeth Hospital, Woodville, Australia.

The inflammatory bowel diseases, consisting of Crohn's disease and ulcerative colitis are amongst the most common complex diseases, with an estimated combined prevalence of 200 per 100,000. Genome scans of patient groups from around the world have implicated more than 5 separate genetic localisations, with one, the IBD1 locus on chromosome 16, having been confirmed in a large international collaborative effort with a combined multipoint LOD score of 5.79. The IBD1 gene has recently been characterised, with the 3020insC frameshift being implicated as the major causal mutation in European and American populations. This mutation occurs at approximately twice the frequency in affected individuals as that in normal individuals. Linkage evidence has offered variable support for the localisation in those populations, with the MLS varying from 3.4 to 2.5. In the Australian population however, an MLS of 6.3 has been reported, suggesting that this disease gene and its mutations may be of greater importance for the expression of the phenotype in Australian patients. Using over 200 Australian multiplex IBD families, more than 100 sporadic cases, and over 100 normal individuals, we show that the IBD1 frameshift mutation is extremely common in Australian CD. The allele frequency in familial CD probands is 0.14, but only 2% of probands are homozygous for the frameshift. The allele frequency is significantly lower in the sporadic form of the disease (0.026) which is nevertheless X 3.7 that observed in the normal population. This is in contrast to the allele frequencies reported in European and American populations. We relate these data to the linkage evidence, and also to disease type and age of onset data.

**Association of a conserved promoter haplotype in the 5-lipoxygenase gene with reduced levels of peripheral eosinophils in Chinese patients with asthma.** *G. Barnes, E. Nolin, S. Lewitzky, J. Shanahan, A. Aelony, J. Roach, J. Meyer.* Millennium Pharmaceuticals Inc, Cambridge, MA.

The product of the 5-lipoxygenase (5LO) gene is involved in the first two steps of the metabolism of arachidonic acid into leukotrienes. The involvement of 5LO in the etiology of asthma is demonstrated by the fact that inhibitors of 5LO are useful in the treatment of asthma. We have scanned the entire 5LO gene for nucleotide variants including the exonic regions, intronic sequences flanking each exon, 5 and 3 UTRs, and the upstream regulatory region. This scan revealed a 6 marker haplotype in the promoter region of 5LO that is conserved at a relatively equal frequency across three distinct ethnic backgrounds (approximately 0.18 in Caucasian, Asian Chinese, and African American individuals). The haplotype consists of four nucleotide substitutions, one insertion/deletion variant, and one previously described polymorphic SP1 binding motif. Five of the variants (the four SNPs and the insertion/deletion) were estimated to be in complete linkage disequilibrium across all three ethnic groups studied. A non-wildtype allele of 4 SP1 repeats (4 allele) resides on the haplotype in Caucasians and Asian Chinese individuals. In the case of African Americans who carry the haplotype, a non-wildtype allele appears to reside on the haplotype but not necessarily the 4 allele. Analysis of this haplotype in a large family collection of Chinese patients with asthma reveals that this haplotype is associated with reduced levels of peripheral eosinophils.

**Putative role for the calpain 10 gene in free fatty acid metabolism.** *M. Orho-Melander, M. Klannemark, M. Svensson, C.M. Lindgren, L. Groop.* Department of Endocrinology, Lund University, Malmö, Sweden.

Intronic variation in the gene coding for the cysteine protease calpain 10 (CAPN10) on chromosome 2q (NIDDM1) has been associated with type 2 diabetes, but information on molecular and physiological mechanisms explaining these associations are limited. Here we addressed this question by studying the role of variants in CAPN10 for phenotypes associated with the metabolic syndrome (MSDR) and FFA metabolism using both a case-control and family-based association study design. Of the genotyped SNPs -43, -44, -19 and -63, the SNP -43 (allele1; 75.5 vs. 69.4%;  $p=0.011$ ) and -63 (allele 2; 11.3 vs. 7.1%;  $p=0.010$ ) as well as haplotypes SNP-43/19/63 (121/121 or 121/112; 17.5 vs. 9.5%;  $p=0.0026$ ) and SNP-43/44 (11/11; 31.6 vs. 20.9%;  $p=0.0017$ ) were associated with type 2 diabetes in 394 type 2 diabetes patients and 297 nondiabetic controls from Finland. 10% of the control subjects and 66% of patients with type 2 diabetes were classified as having MSDR. Allele frequencies of SNP-43 and -63 were similar between type 2 diabetes patients (with or without MSDR) and controls with MSDR (75.5 vs 75.0% and 11.3% vs. 13.8%), while there was a significant difference compared to controls without MSDR (75.5 vs. 68.7%,  $p=0.0056$  and 11.3 vs. 6.4%,  $p=0.0021$ ). Among the control individuals, the SNP-43 genotype 22 was associated with lower fasting insulin ( $6.8\pm 3.4$  vs  $8.5\pm 4.9$  mU/l,  $p=0.021$ ), lower HOMA insulin resistance index ( $1.6\pm 0.8$  vs  $2.1\pm 1.3$ ,  $p=0.0076$ ) and lower fasting free fatty acids, FFA ( $522\pm 186$  vs  $689\pm 233$  mmol/l,  $p=0.0040$ ). The 22 genotype was also associated with lower FFA in the whole study group ( $558\pm 189$  vs  $779\pm 290$  mmol/l,  $p=0.00020$ ), particularly among males ( $473\pm 154$  vs  $697\pm 277$  mmol/l,  $p=0.0010$ ). None of the four SNPs showed distorted transmission of alleles to patients with type 2 diabetes in TDT including 108 trios. However, sex, age and BMI adjusted quantitative TDT revealed association between the SNP-44 and fasting serum insulin ( $p=0.049$ ). In conclusion, the CAPN10 gene is associated with type 2 diabetes, features of insulin resistance and FFA levels. Our results suggest a role for CAPN10 in fatty acid metabolism.



**Novel Myocilin Mutations in West African Individuals with Primary Open Angle Glaucoma.** *K.R. LaRocque<sup>1</sup>, R.R. Allingham<sup>1</sup>, M.A. Hauser<sup>1,2</sup>, L.W. Herndon<sup>1</sup>, B.W. Broomer<sup>1</sup>, M.A. Pericak-Vance<sup>1,2</sup>, P. Challa<sup>1</sup>.* 1) Department of Ophthalmology; 2) Department of Medicine, Section of Medical Genetics, Duke University Medical Center, Durham, NC.

We have investigated the role of myocilin mutations in a West African primary open angle glaucoma (POAG) population to determine if there is a correlation between the observed phenotype and mutations in myocilin. POAG patients with mutations in myocilin have been shown to have an earlier age of onset as well as a more severe clinical presentation, mirroring the phenotype found in POAG patients in West Africa. POAG patients and controls were recruited at the Emmanuel Eye Clinic in Accra, Ghana. Ascertainment criteria for POAG were age of diagnosis >25 yo and two out of three of the following criteria: 1) applanation IOP  $\geq 22$  in each eye, 2) glaucomatous optic neuropathy OU including cup-to-disc ratio  $\geq 0.7$ , optic disc asymmetry  $\geq 0.2$ , or notching or focal thinning of the optic disc rim, 3) visual field loss measured by modified tangent screen or Humphrey field testing consistent with glaucoma in at least one eye. Age matched unaffected controls were obtained in patients with an IOP <22 mmHg and normal appearing optic nerves. Ninety unrelated affecteds with POAG and 76 control patients were recruited. PCR amplification was performed on each of the three myocilin exons, followed by denaturing high performance liquid chromatography to detect allelic differences. Samples demonstrating a polymorphism were sequenced on both strands. Four individuals with severe POAG were found to have novel missense mutations in exon 3. Two exhibit an Asp380Asn mutation and two an Arg342Lys mutation. These changes were not detected in 152 ethnically matched control chromosomes. Fourteen affected individuals and 8 controls exhibit a translationally silent polymorphism in codon 325 (Thr325Thr). Four percent of the study patients were found to have novel disease-associated mutations in myocilin, which is consistent with the percentage found in other populations. Therefore, mutations in myocilin appear to play a limited role in the pathogenesis of POAG in this region of West Africa.

**An MAOA\_VNTR and Childhood Behavior Disorders.** *D.C. Rowe<sup>1</sup>, S. Black<sup>1</sup>, M. Gilson<sup>1</sup>, H. Brown<sup>2</sup>, A. Abramowitz,<sup>3</sup> S. Sherman<sup>4</sup>, I.D. Waldman<sup>5</sup>.* 1) Div Family Studies/Human Dev, Univ Arizona, Tucson, AZ; 2) Graduate Program in Genetics, University of Arizona, Tucson; 3) Department of Psychiatry, Emory University, Atlanta, GA; 4) Department of Genetics and Molecular Medicine, Emory University; 5) Department of Psychology, Emory University.

The MAOA\_VNTR is a 30 bp repeat polymorphism in the MAOA gene promoter region. The polymorphism is functional, with greater gene expression associated with the A3 allele than with the A1 allele (two rare alleles were grouped with the common ones by their gene expression levels). The protein product of this gene degrades neurotransmitters, preferentially serotonin and norepinephrine. In one prior study, the A3 allele has been associated with a higher level of self-reported aggression in a community sample. We examined the association between the A3 allele and attention deficit disorder (ADHD), hyperactive impulsive disorder (HID), and attention deficit disorder (ADD), diagnosed from symptoms in parental questionnaire reports on their children. The subjects were 156 male children, mean age 9.8 years ( $s = 2.9$  years), divided between clinic referred children and their siblings and control (non-referred) children and their siblings. The allele frequencies were  $A3 = .57$ ,  $A1 = .43$ . Chi square tests comparing the diagnosed boys against boys in the control group yielded no significant differences ( $P > .30$ ). Within-family tests were used to compare the frequency of transmission of the A3 allele vs. that of the A1 allele using informative, heterozygous mothers (fathers being irrelevant because MAOA resides on the X chromosome). These within-family tests were also negative. Thus, this polymorphism in MAOA is probably not affecting the risk of these childhood psychiatric disorders.

**An atypical case of Angelman Syndrome caused by an imprinting defect.** *F.E. Tahmaz, M.A. Kalichman, G.E. Hoganson, F. Quan.* Dept Pediatrics, Div Genetics, Univ of Illinois at Chicago, Chicago, IL.

Angelman Syndrome (AS) is characterized by severe mental retardation, absent speech, seizures, ataxia, inappropriate laughter, and microcephaly. AS is caused by the loss of maternally expressed genes from the imprinted chromosome 15q11-q13 region. A small number of AS patients with an atypical phenotype that includes features of Prader-Willi Syndrome (PWS) (obesity, hypotonia, mild mental retardation) have recently been reported (Gillissen-Kaesbach et al, 1999). In these patients, the maternally-derived SNRPN allele was absent or reduced. We present here an additional case in which a child with a methylation pattern at the SNRPN gene consistent with AS had an atypical phenotype for AS. The patient was a 3 year-old male with severe mental retardation, absent speech, microcephaly (head circumference at 2nd centile), height >95th centile, and obesity (weight 52 lbs, > 95th centile). High resolution chromosomes and Fragile X Syndrome testing were normal, as were a urine organic and amino acid screen. The patient was not dysmorphic and did not have the typical phenotypic characteristics of AS or PWS. However, since the patient's weight had almost doubled over the period of a year, a methylation study for PWS was performed. Methylation-specific PCR of the SNRPN gene revealed that the patient had not inherited a maternally imprinted SNRPN allele, a result that was consistent with AS. The absence of a maternally imprinted SNRPN allele was confirmed by Southern analysis using an XbaI-NotI double digest and a probe specific for the 5'-end of the SNRPN gene. No maternal band was observed. FISH analysis using a probe for the SNRPN gene showed that the patient was negative for a 15q11-q13 deletion. Microsatellite analysis using the markers D15S541, D15S113, GABRB3, and D15S115 was consistent with biparental inheritance of chromosome 15, indicating that the abnormal methylation pattern in this patient was the result of an imprinting defect. The atypical phenotype of our patient may be the result of cellular mosaicism for the imprinting defect or alternatively, a partial imprinting defect.

**A Low Density Lipoprotein Receptor Related Protein-Associated Protein 1 Del/Ins Polymorphism and Phenotypic Variability in Alzheimer Disease.** *D.L. Schutte<sup>1</sup>, M. Maas<sup>1</sup>, K.C. Buckwalter<sup>1</sup>, J.C. Murray<sup>2,3</sup>, B.C. Schutte<sup>2,3</sup>.* 1) Col of Nursing, Univ Iowa, Iowa City, IA; 2) Dept of Pediatrics, Univ Iowa, Iowa City, IA; 3) Genetics PhD Program, Univ Iowa, Iowa City, IA.

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by dementia. AD exhibits individual variability in age at onset, rate of progression, and specific cognitive, functional, and behavioral features. Genetic variants are potential modulators of phenotypic variability. The purpose of this study was to explore the relationship between variations within six candidate susceptibility loci, including the Low-Density Lipoprotein Receptor Related Protein-Associated Protein (LRPAP1) gene (4p16.3), and repeated measures of cognition, function, and behavior in persons with AD, using outcome data collected in two caregiver intervention studies. Thirty-seven subjects diagnosed with probable or possible AD, who participated in either the Family Involvement in Care Study (PI: Maas) or the Progressively Lowered Stress Threshold Study (PI: Buckwalter), were recruited. Subjects exhibited a mean age at onset of 68.7 years (S.D.=9.08); 92% of the sample were Caucasian. The results of the LRPAP1 analyses are reported here. All subjects were genotyped for a 37bp del/ins polymorphism in intron 5 of the LRPAP1 gene. No differences in allele or genotype frequencies by gender or by age at onset were identified. No statistically significant genotype effects upon cognition or behavior were identified. However, trends were noted in measures of memory and language. The LRPAP1 ins-positive subjects exhibited poorer memory scores (average score difference=8%,  $p=.103$ ) and poorer language scores (average score difference=28%,  $p=.158$ ). LRPAP1 ins-positive subjects were significantly more functionally impaired than subjects without the LRPAP1 insertion allele ( $F_{1,7}=7.36$ ,  $p=.030$ ). These results suggest genetic variations at the LRPAP1 locus modulate AD phenotype beyond risk for disease. Potential genotype-phenotype relationships were identified that require analyses with a larger sample representing the full AD phenotype trajectory.

**Renin-angiotensin system (RAS) DNA polymorphisms and TGF $\beta$ 1-mediated fibrogenic pathway gene expression studies in primary IgA nephropathy (Berger's disease).** A. Turco<sup>1</sup>, C. Patuzzo<sup>1</sup>, M. Brugnara<sup>1</sup>, C. Marcantoni<sup>2</sup>, B. Brezzi<sup>2</sup>, A. Lupo<sup>2</sup>, G. Gambaro<sup>3</sup>, F. Anglani<sup>3</sup>, D. Del Prete<sup>3</sup>, G. Ligabue<sup>4</sup>, L. Furci<sup>4</sup>, R. Magistroni<sup>4</sup>, A. Albertazzi<sup>4</sup>, G. Maschio<sup>2</sup>. 1) Department of Mother & Child, Section of Genetics, University of Verona School of Medicine, Verona, Italy; 2) Division of Medical Nephrology, University of Verona, Italy; 3) Dept of Medical & Surgical Science, Nephrology Division, Univ of Padova, Padua, Italy; 4) Dept of Internal Medicine, Nephrology Division, Univ of Modena, Italy.

Primary IgA nephropathy (IgAN) is the most common human nephropathy and a leading cause of end stage renal disease (ESRD). DNA RAS polymorphisms (RASPs) have been implicated as genetic susceptibility factors in IgAN development and progression towards ESRD. 90 Italian biopsy-proven IgAN patients are being recruited and genotyped for the following gene RASPs: I/D angiotensin converting enzyme (ACE); A1166C, angiotensin II receptor(AT1); and M174T, angiotensinogen (AGT). 100 healthy blood donors were used as normal controls. Comparative RT/PCR was also used in microdissected kidney biopsies to assess glomerular and interstitial mRNA levels of the RAS and the fibrogenic pathway (TGF $\beta$ 1, collagen III and IV, a smooth muscle actin). ACE and AT1 genotype frequencies did not differ significantly between IgAN and control populations. Unexpectedly, glomerular ACE mRNA levels were significantly higher in pts with the ACE-II genotype. No AGT-MM genotypes and a significant higher prevalence of AGT-TM genotypes (T174M) were detected in IgAN pts. Higher M235T TT and lower MM genotype distributions were also observed in the IgAN population. These preliminary results differed significantly from the expected genotype frequencies, given the remarkable under representation of the T174M-MM genotype in IgAN pts. In our pt population the AGT-T allele seems to confer susceptibility to IgAN development. We also assessed whether the AGT T174M polymorphism influenced the relationships between glomerular AGT and fibrogenic pathway gene expression. We found that the MT genotype strongly influenced this correlation. Overall, our preliminary data point to a possible involvement of the AGT T allele in IgAN.

**Transforming growth factor beta-1 (TGF- $\beta$ 1) SNPs are associated with progressive renal insufficiency.** *M. Salah*<sup>1</sup>, *A.M. El Nahas*<sup>2</sup>, *A.I.F. Blakemore*<sup>3</sup>. 1) Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK; 2) Sheffield Kidney Institute, Northern General Hospital, Sheffield, UK; 3) Department of Medical and Community Genetics, Kennedy-Galton Centre, Imperial College of Science, Technology and Medicine, London, UK.

TGF-1 plays an important role in the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis. Cytokine and growth factor SNPs are receiving increasing interest in association studies with disease entities, including nephropathies. In many cases, genetic associations with cytokine genes affect the clinical course of diseases, rather than simply being susceptibility factors. In this study, we tested TGFB1 gene polymorphisms as possible markers for the progression of chronic renal failure (CRF). We screened 142 Caucasian patients with CRF for TGFB1 SNPs: LEU10PRO, ARG25PRO, C76T and T-509C. 100 ethnically-matched control subjects were also genotyped. We found no significant differences between patient and control groups in allele frequencies or carriage rates, indicating that the markers tested did not affect susceptibility to CRF. We observed a significant association between genotype at codon 25 (odds ratio 5.8, 95%CI 1.7-19.4,  $p < 0.001$ ) and at -509 (odds ratio 2.85, 95%CI 1.2-6.6,  $p < 0.005$ ) with the rate of progression of CRF (slope of 1/creatinine) in patients with primary nephropathies (PKD excluded). Also, an association was found between ARG25PRO and the severity of proteinuria ( $p = 0.046$ ). Alleles at codon 25 and -509 exhibit strong linkage disequilibrium. In immunohistochemistry studies of biopsies from patients with CRF, TGFB1 SNPS were associated with differences in local TGF-1 protein levels and with degree of inflammatory infiltrate. TGFB1 markers may, therefore, be useful as prognostic indicators for the progression of CRF.

**Increased prevalence of thrombophilia and hypofibrinolysis abnormalities in patients with Chronic Fatigue**

**Syndrome: Identification of probable predisposition factors.** *H.H. Harrison<sup>1,2</sup>, D.E. Berg<sup>1</sup>, L.H. Berg<sup>1</sup>, J. Brewer<sup>3</sup>.*

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Recent data have shown low level activation of coagulation in patients with active Chronic Fatigue Syndrome and/or Fibromyalgia (CFS/FM) [Ref: Blood Coag Fibrinol 10:435-438]. To investigate a predisposing association with genetic abnormalities in thrombophilia and/or hypofibrinolysis we analyzed antithrombin (AT), protein C (PC), protein S (PS), activated protein C resistance (APCR), prothrombin (F2), lipoprotein (a) (Lp(a)), and plasminogen activator inhibitor-1 (PAI-1) in 45 patients with clinically-defined CFS/FM, no known thrombosis or interfering systemic disease, and no anticoagulation. Samples were analyzed with functional, antigen or DNA assays and classified as positive or negative against normal reference values. The cohort was also stratified by the ISAC panel of sensitive indicators of low level activation that included fibrinogen (FIB), prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complexes (TAT), soluble fibrin monomer (SFM), and platelet surface P-selectin (CD62P); with positivity defined as 2 or more assays exceeding ref limits. 33 of 45 patients (73%) were ISAC-pos. Significant differences between the total CFS/FM cohort and general population values were observed for F2 levels ( $P < .001$ ), LP(a) levels ( $P < .005$ ), and PAI-1 levels ( $P < .001$ ); with abnormality frequencies for F2 (0.422), Lp(a) (0.333), and PAI-1 (0.267). PAI-1 was the only marker that was different ( $P < .01$ ) in the ISAC-pos group (0.294) versus the ISAC-neg group (0.150). These results show increased prevalence of abnormalities in inherited thrombophilia and/or hypofibrinolysis factors that yield probable genetic predisposition in patients who develop CFS/FM following acute or chronic infections that damage vascular endothelium. Abnormality rates are similar to patients with thrombotic histories and suggest a potential need for monitoring CFS/FM patients for clinical thrombosis. These results are also consistent with family histories related to CFS/FM and suggest assays useful for future family studies.

**Microarray analysis of a cell culture model of familial Parkinson's Disease, linked to  $\alpha$ -synuclein.** *M.J. Baptista, M.R. Cookson, C. O'Farrell, L. Vink, M. Farrer.* Neurogenetics, Mayo Clinic, Jacksonville, FL., USA.

Parkinsons Disease (PD) is an age related neurodegenerative condition that involves damage to the nigrostriatal dopamine system. The etiology of PD is unclear, but a small number of families show Mendelian inheritance. Point mutations in the gene encoding  $\alpha$ -synuclein, a synaptic protein, have been shown to be associated with autosomal dominant PD. Several studies have identified other regions of linkage in familial PD cases for which the genes in question have not yet been identified. The aim of the current study was to use a cell culture model of synuclein over-expression to identify additional genes relevant to PD.

We have used the oligo-based U95A GeneChip (Affymetrix, Santa Clara, CA) to globally compare the expression levels of 12,500 non-redundant cDNAs and ESTs in stable clonal neuroblastoma cell lines over-expressing either wild type  $\alpha$ -synuclein or one of the disease-associated mutations (A53T). Forty-five genes were identified that gave both (1) a change of at least 2-fold compared to control cell lines and (2) a strong positive probe hybridization. The microarray data was confirmed by quantitative RT-PCR using SYBR green as a reporter. Six of the genes identified encoded transcription factors, having a 5.7 net fold increase in expression in cells over-expressing A53T synuclein as compared to those over-expressing wild type  $\alpha$ -synuclein. Other pathways of interest may include apoptosis, as we have seen a 2-fold down-regulation of the mRNA for caspase-3, a pro-cell death protease. Likewise, we have noted a reduction in expression of the nicotinic acetylcholine receptor alpha 5 subunit, which is known to modulate dopamine release. Some mRNA species were up-regulated in cells expressing mutant synuclein and unchanged in cells over-expressing wild type synuclein, such as the iron responsive binding protein aconitase. It is expected that identification of additional gene families regulated by over-expression of synuclein will form a pathway leading to PD, the identification of which is an important objective in PD research.



**Two new mutations in the CD45 gene in multiple sclerosis patients.** *M. Gomez Lira*<sup>1</sup>, *D. Bonamini*<sup>1</sup>, *A. Salviati*<sup>2</sup>, *PF. Pignatti*<sup>1</sup>. 1) Dept Mother-Child Biol Genetic, Sect Biol-Genetics, Verona, Italy; 2) Dept of Neurological Scienze, Verona, Italy.

Multiple sclerosis (MS) is a chronic disabling disease of the central nervous system characterized by T cell mediated destruction of the myelin. MS is held to be a complex autoimmune disease with a strong genetic component. Inflammation and demyelination are involved in the pathology, indicating that genes influencing the immune system can be implicated in the ethiology of MS. Recently an association between a variant CD45 isoform expression and multiple sclerosis has been observed in case-control studies, (Nature Genetics 2000, Vol 26; 495-99). CD45 is a cell surface protein-tyrosine phosphatase, which is believed to be a critical regulator of lymphocyte activation. It is a high molecular weight glycoprotein with multiple isoforms as a result of alternative splicing. CD45 negatively regulates JAK-STAT signaling in response to multiple cytokines, and genetic inactivation of CD45 in cells enhanced cytokine-triggered proliferation, differentiation and antiviral activities. The expression of a variant isoform of CD45 observed in MS patients is due to a point mutation in exon 4 (C/G at nucleotide 77), which prohibits out splicing of exon 4, leading to strong expression of high molecular weight CD45 isoforms. Other mutations on the CD45 gene could have a similar effect upon protein or protein expression and could be associated to MS susceptibility. In this study we screened 57 MS patients and 62 sex matched control individuals for the C77G in the CD45 gene. The mutation was present in heterozygosity in three MS patients and in one control individual. The difference between MS patients and control individuals is not significant. Other mutations in exon 4, 5, and 6, of the CD45 gene were investigated by screening amplified fragments including exons and exon/intron boundaries by heteroduplex analysis and direct sequence. We have identified two new CD45 gene mutations. A C/T transition at position 77 in exon 4, present in one MS patient, and a G/C transversion at position 69 in exon 5, present in two MS patients. These mutations were not present in 62 control individuals.

**Identifying and characterizing the diabetogenic gene, *iddm4*, in the BioBreeding Diabetes-Resistant rat (BB-DR).**

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Type 1 diabetes mellitus (T1DM) is a multifactorial autoimmune disease in both humans and BioBreeding (BB) rats. Diabetes prone (DP) BB rats, but not diabetes resistant (DR) BB rats, develop T1DM spontaneously. T1DM can be induced in 95% of DR-BB rats. Genetic factors implicated in the control of T1DM include both major histocompatibility complex (MHC)-linked and non-MHC-linked genes. At least two non-MHC loci are linked to the expression of T1DM in the BB rat. *Iddm4* is one susceptibility locus. Spontaneous disease requires homozygosity for the mutation causing lymphopenia (*lyp/iddm1*). We have previously mapped *iddm4* to rat chromosome four. *Iddm4* controls insulinitis and diabetes in both DR and DP-BB rats and is likely to be responsible for the generation of autoreactivity. The current goal is to identify the *iddm4* gene and characterize its mechanism of action. Toward this end, we are constructing an *iddm4* speed congenic rat strain. At each generation of breeding we identify new genetic recombinants and determine the ability of the congenic rats to develop T1DM or to transfer it to immunodeficient histocompatible recipients. The *iddm4* interval currently spans 3cM. To identify the *iddm4* gene, we are evaluating genes and expressed sequence tags (ESTs) that map to the *iddm4* interval. We expect that the *iddm4* allele be shared by both BB substrains and not by WF. We are screening these genes and ESTs in BB, WF, and congenic rats for single nucleotide polymorphisms (SNPs) by conformation sensitive gel electrophoresis (CSGE) and for expression differences by slot blot array and real-time PCR. To date, 62 ESTs have been screened by CSGE and 30 have been evaluated by slot blot array. We identified five polymorphic ESTs; these ESTs serve as markers to fine map the interval. None of the 30 ESTs evaluated by slot blot array was found to exhibit a significant change in expression. Expression of candidate genes and ESTs in the 3cM *iddm4* interval is currently being evaluated by real-time PCR.

**Functional analysis of polymorphisms in the promoter region of glutamate receptors and transporter genes.** C.A. Guy, K. Smith, B. Hoogendoorn, S. Coleman, P.R. Buckland, M.C. O'Donovan. Psychological Medicine, UWCM, Cardiff, Wales.

We have searched the promoter regions of glutamate receptors and transporters for common polymorphisms and analysed these for functional effect on gene transcription in a reporter gene assay. As well as being associated functionally with a number of physiological processes, glutamatergic neurotransmission has also been associated with certain pathophysiological processes, including schizophrenia. We have screened the first 500 bp of the promoter region of 25 glutamate ionotropic receptor subunits, 8 metabotropic receptors and 6 transporters for common polymorphisms using denaturing high performance liquid chromatography. A total of 16 promoters were found to contain polymorphisms which were confirmed and characterised by sequencing. Each allele pair was cloned into a modified pGL3 luciferase reporter gene vector, characterised by DHPLC and sequencing, and used to transfect each of three human cell lines (HEK293t, TE671 and JEG-3) along with pCMV-SPAP as an internal control. The relative promoter activity of allele pairs was measured. Several polymorphisms caused significant changes to promoter activity.

**No missense mutation of *WKL1* in a subgroup of probands with schizophrenia.** *J.M. Devaney<sup>1</sup>, E.A. Donarum<sup>1</sup>, K.M. Brown<sup>1</sup>, J. Meyer<sup>2</sup>, K-P. Lesch<sup>2</sup>, D.A. Stephan<sup>1</sup>, A.E. Pulver<sup>3</sup>.* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, D.C; 2) Department of Psychiatry and Psychotherapy, University of Würzburg, Germany; 3) Johns Hopkins University School of Medicine, Epidemiology-Genetics Program in Psychiatry, Baltimore, Maryland.

Recently, a Leu309Met mutation in *WKL1*, a gene mapped to chromosome 22q13.33, was reported to co-segregate with periodic catatonia, a clinical sub-type of schizophrenia, in 7 members of an extended pedigree<sup>1</sup>. *WKL1* encodes a putative non-selective cation channel expressed exclusively in the brain, particularly in the amygdala, nucleus caudatus, thalamus, and hippocampus<sup>1</sup>. We screened *WKL1* for etiologic mutations in 28 probands from the United States who were given a consensus diagnosis of schizophrenia and met at least one of these criteria: 1) were from multiplex schizophrenia families where at least two schizophrenic subjects were reported to display catatonic behavior at sometime during the course of their illness, or 2) were from multiplex schizophrenia families where, in a genome scan for schizophrenia susceptibility loci, evidence for excess allele sharing among affected family members for markers in the 22q13 region was seen. In addition, 15 subjects from the 12 original German pedigrees in the Meyer et al. report were similarly screened for causative mutations. This German cohort exhibited the catatonia phenotype but had ambiguous linkage to 22q13 and included the mutation-positive proband as a positive control. The 43 probands were screened for base changes in *WKL1*: 15 SNPs in the non-coding regions of the gene, three SNPs in the 3'UTR, four synonymous coding SNPs and two non-synonymous (amino acid changing) SNPs were identified. We were able to rapidly confirm the Leu309Met nucleotide change in the positive control. No missense mutations were detected in any of the other 42 probands studied. These data exclude the role of *WKL1* in the subjects studied and can be interpreted as providing further evidence of the genetic heterogeneity of schizophrenia.

**A two-stage association study between inflammatory bowel disease and polymorphisms spanning GNAI2.** *B.M. Dechairo*<sup>1,2</sup>, *N. Lench*<sup>1</sup>, *D. van Heel*<sup>3</sup>, *M. Edwards*<sup>1</sup>, *P. Scambler*<sup>2</sup>, *D.P. Jewell*<sup>3</sup>, *A.H. Carey*<sup>1</sup>. 1) Oxagen Ltd, Abingdon, England; 2) Molecular Medicine Unit, Institute of Child Health, University College of London, London, England; 3) Gastroenterology Unit, Nuffield Department of Medicine, Radcliffe Infirmary, Oxford, England.

Inflammatory Bowel Disease (IBD) includes ulcerative colitis (UC) and Crohns disease (CD), two chronic inflammatory diseases of the intestine. Aetiology is unknown but may involve a complex interaction between genetic susceptibility and environmental factors. Recently, several IBD susceptibility loci have been identified by linkage studies. Of these loci, one on chromosome 3p has been detected in two genome scans and a subsequent, region-specific, linkage study. To further analyse this region, we examined an IBD candidate gene within the region, guanine nucleotide-binding protein alpha i2 (GNAI2), for association in a population of 333 IBD European simplex families. In the first stage of the association study, four single nucleotide polymorphisms (SNPs) identified by denaturing high-performance liquid chromatography (dHPLC), were genotyped across a sub-group of 185 families. The SNPs were spaced every 6 kb spanning the genomic region of GNAI2. In the second stage of the association study, only the two flanking SNPs were genotyped in the remaining families due to almost complete marker-marker linkage disequilibrium (LD) between three of the SNPs. Association analysis was performed using the TDT functions implemented in GENEHUNTER 2.0. Positive association was seen with the three SNPs ( $p = 0.004$ ,  $0.02$ , and  $0.007$ ) in LD and with a haplotype of alleles from all four SNPs ( $0.007$ ) in 58 CD families used in the first stage. However, no association was seen in the 156 CD families analysed after the second stage. No association was seen in either stage of the study for both IBD and UC. With no discernable difference in the collection criteria and ethnicity of the CD families used for both stages of the study, the analysis of the combined dataset may be a better reflection of the IBD association to GNAI2. Thus, GNAI2 does not appear to be an IBD susceptibility gene.

**Ethnic specific differences in Nod2 associations to Crohn's disease.** *D.K. Bonen<sup>1</sup>, Y. Ogura<sup>2</sup>, R.H. Duerr<sup>3</sup>, S.R. Brant<sup>4</sup>, G. Nuñez<sup>2</sup>, J.H. Cho<sup>1</sup>.* 1) Medicine, University of Chicago, Chicago, IL; 2) Pathology, University of Michigan, Ann Arbor, MI; 3) Medicine, University of Pittsburgh, Pittsburgh, PA; 4) Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Crohn's disease (CD) is a common complex disorder affecting young adults and is associated with higher disease prevalence in Ashkenazi Jews than in non-Ashkenazi Caucasians. We have established that three coding region polymorphisms (Leu1007fsinC, Gly908Arg, Arg702Trp) in Nod2 are highly associated with CD in an American cohort (Nature 411:603, 2001). The Arg702Trp variant is associated with the highest population attributable risk among non-Ashkenazi Caucasians. Functional proof of causation has been established for the frameshift mutation, which shows a marked hyporesponsiveness (NF- $\kappa$ B activity) to bacterial LPS. However, the functional variation associated with Gly908Arg and Arg702Trp is much less dramatic, and their direct role in disease pathogenesis is not established. We report data on novel SNPs and STRs in and near Nod2 that are common to the CD associated variants. Very distinct patterns of inheritance are observed, with Arg702Trp, Gly908Arg, and Leu1007fsinsC occurring on a similar haplotypic background. While Leu1007fsinsC has comparable allele frequencies in non-Ashkenazim and Ashkenazim, Gly908Arg is significantly more frequent among Ashkenazim (10.2%) compared to non-Ashkenazim (4.3%). Furthermore, Gly908Arg demonstrates preferential transmission in both subsets. Given the functional significance of amino acid polymorphisms in the LRR and the consistency of ethnic associations observed for the Gly908Arg variant, its direct role in CD pathogenesis is likely. Arg702Trp has a significantly lower allele frequency among Ashkenazim (2.2%) compared to non-Ashkenazim (10.8%), with an inverse transmission observed among the Ashkenazim. Since Arg702Trp is not located in the LRR of Nod2, it is possible that it is not directly causative, but is in linkage disequilibrium with the causative variant. Consideration of ethnic specific haplotype associations will assist in defining the precise causative variant accounting for the observed association of Arg702Trp to CD.

**A putative functional polymorphism in the promoter of the prodynorphin gene is associated with cocaine dependence or abuse.** *A.C.H. Chen<sup>1</sup>, K.S. LaForge<sup>1</sup>, A. Ho<sup>1</sup>, P.F. McHugh<sup>1</sup>, S. Kellogg<sup>1</sup>, K. Bell<sup>1</sup>, R.P. Schluger<sup>1</sup>, S.M. Leal<sup>2</sup>, M.J. Kreek<sup>1</sup>.* 1) The Laboratory of Biology of Addictive Diseases; 2) The Laboratory of Statistical Genetics, The Rockefeller University, New York, NY.

The opioid peptide dynorphin has been demonstrated to play a role in modulating neurochemical responses to cocaine. Our laboratory and others have found that acute or chronic exposure to cocaine increases dynorphin gene expression in rats. Recently, a polymorphic 68-bp repeat within the promoter region of the human prodynorphin gene has been identified (Zimprich et al., 2000, J Neurochem.). This repeat is present in one, two, three, or four copies and contains a putative AP-1 transcription factor binding site. Previous in vitro evidence shows that alleles containing three or four copies are associated with an increase in stimulated promoter activity. We hypothesize that the 3 and 4 repeat alleles may be protective against cocaine dependence or abuse through a dynorphin-mediated mechanism. From a study of the genetics of addiction, 174 subjects were studied: controls with no history of substance abuse or dependence (n=91) and cases with a primary diagnosis (DSM-IV criteria) of cocaine dependence (n=61) or abuse (n=22). Primers were designed for polymerase chain reaction (PCR) to amplify the region of the prodynorphin gene containing the repeat element. All PCR products were sized on agarose gels and twenty samples were verified by direct sequencing. The association of cocaine dependence or abuse with alleles containing three or four repeats was examined. With data stratified by ethnicity, pooled Relative Risk with Mantel-Haenszel Chi square was calculated: Relative Risk = 0.59 (95% confidence interval 0.37 - 0.95),  $c^2_{(1)} = 4.14$ ,  $p = 0.042$ . Our results suggest that the 3 or 4 repeat alleles may confer a protective effect against cocaine dependence or abuse.

**Identification of a novel transition period in non-obese diabetic (NOD) mice by cDNA microarray.** *S.E.*

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Type 1 diabetes is an autoimmune disease that afflicts more than 1 million Americans. In this disease, the insulin-producing beta cells of the pancreas are targeted for destruction by the body's own immune system. Two categories of genes are of great interest in type 1 diabetes: the genes that cause the disease and those that can be used to predict individuals at high risk for developing the disease. Microarray technology was employed to identify genes in both non-obese diabetic (NOD) mice, a mouse model that spontaneously develops diabetes, and NOD control strains, NOD.Idd3/Idd10 and NOD.B10 (<1% and 0% incidence of diabetes, respectively). A splenic cDNA library containing 11,520 clones was used to perform microarrays on the spleens of individual NOD and control mice at time points from 1 to 10 weeks of age. Data analysis of these microarrays revealed a transition period between 3 weeks and 6 weeks in the NOD mice. Sequencing the top 1,000 clones generated a list of 180 unique genes. Of the 48 genes that are up regulated, 12 are B cell related suggesting an expansion of the B cell population or sub-population in the NOD mice that is not seen in the control strains. Flow cytometry performed on splenic lymphocytes from NOD and NOD control mice at 3 and 6 weeks of age reveal that 3 week NOD mice have fewer CD21<sup>+</sup> B cells than either 6 week NOD or either control strains at 3 or 6 weeks of age. Further studies of the B cell population during this critical transition period will aid in our understanding of the diabetogenic process and help identify genes that may be used as molecular markers for disease prediction.



**Promoter region variants in G protein receptor kinase-3 (GRK3) are associated with bipolar disorder.** *T.B.*

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In a genome-wide linkage survey we have previously shown evidence indicating chromosome 22q12 contains a susceptibility locus for bipolar disorder (BPD) in the vicinity of GRK3. GRK3 is an excellent candidate risk gene for BPD since GRKs play key roles in the homologous desensitization of G protein-coupled receptor signaling. To identify mutations in GRK3 we sequenced the putative promoter region, exons, and flanking intron in 14 individuals with BPD. We found six variants in the promoter/5-UTR region, but no coding or obvious splice variants. Transmission disequilibrium data from two triad sets indicate two of the promoter/5-UTR variants, found as a linked haplotype, are associated with BPD in families of Northern European (NE) Caucasian ancestry but not in non-NE Caucasian or non-Caucasian families. In 329 NE Caucasian triads the transmission to non-transmission ratio was 25:7, chi-square = 10.1,  $p = 0.003$ . To confirm promoter activity we have cloned a 1.5 kb genomic region lying immediately upstream of the first coding exon into a luciferase reporter expression vector and transfected this construct into SK-N-MC neuroblastoma cells, demonstrating that this region has significant transcriptional activity. The possibility that the two variants associated with disease are mutations which effect transcription or translation will be studied by transfection of expression constructs into SK-N-MC cells. These data support the hypothesis that a dysregulation in GRK3 expression which alters signaling desensitization contributes to the development of BPD.

**C4AQ0 and C4BQ0 genotyping: association with systemic lupus erythematosus.** *X-Y. Man<sup>1,2</sup>, H-R. Luo<sup>1</sup>, X.P. Li<sup>2</sup>, Y-G. Yao<sup>1</sup>, C.Z. Mao<sup>2</sup>, Y-P. Zhang<sup>1</sup>.* 1) Lab. Cellular Molec. Evol., Kunming Inst. Zool., CAS, Kunming, Yunnan 650223, P.R. China; 2) Department of Dermatology, the Second Affiliated Hospital, Kunming Medical University, Kunming, 650101, China.

Systemic lupus erythematosus (SLE) is a multifactorial complex autoimmune disease characterized by various autoantibodies that recognize autoantigens. The association of C4 deficiency with SLE was previously well documented based on electrophoretic mobility of C4 protein in plasma, but no detailed gene analyses were performed on it. To investigate the association of C4 null genes C4AQ0 and C4BQ0 with SLE in Chinese, we have genotyped 113 patients and 104 normal people. C4 null genes were determined by a PCR procedure with sequence-specific primers (PCR-SSP). To further characterize whether the molecular basis of the defective C4A genes is due to 2bp insertion in exon29, which was previously identified in non-Chinese populations, we designed a pair of exon-specific primers to amplify the whole exon29 followed by direct DNA sequencing. The SLE patients' disease activity and damage were assessed using SLEDAI and SDI, respectively. Statistical analyses were carried out using SPSS software. The frequency of C4AQ0 allele is 15.04% (17/113) in SLE patients compared with 1.92% (2/104) in controls ( $P < 0.001$ ). There was no significance of C4BQ0 allele in SLE patients compared with the controls ( $P = 0.233$ ). SLE patients with C4AQ0 gene had an increased risk of acquiring serositis and arthritis compared with the other patients [For serositis, odds ratio (OR) 4.2, 95% CI 1.2-15.0,  $P = 0.036$ ; For arthritis, OR 2.41, 95% CI 0.64-9.12,  $P = 0.043$ ]. SLEDAI and SDI of SLE patients with and without C4AQ0 gene had no statistically significant difference. In all the 19 individuals with C4AQ0 gene, we didn't detect a 2-bp insertion in exon29 of C4 gene. These results suggested that deficiency of C4A is a risk factor for acquiring SLE in Southwest China, and this results in increased risk of serositis and arthritis in SLE patients. C4AQ0 had no association with SLEDAI and SDI. The genetic basis of C4A nonexpression in Southwest Chinese is not due to 2bp insertion in exon29, and further studies are required to verify it.

**Evidence that MeCP2 mediates transcriptional repression by the AS-IC domain.** *L.B.K. Herzing<sup>1</sup>, S.-J. Kim<sup>2</sup>, L. Villard<sup>3</sup>, E.H. Cook<sup>2</sup>, D.H. Ledbetter<sup>1</sup>*. 1) Dept of Human Genetics, Univ Chicago, Chicago, IL; 2) Dept of Psychiatry, Univ Chicago, Chicago, IL; 3) INSERM U491, Fac.deMedecine LaTimone, Marseilles, FR.

There is considerable phenotypic overlap between autism, Rett syndrome (RS) and Angelman syndrome (AS). We propose that the critical effects of mutations in the methyl-CpG binding protein *MeCP2*, the RS gene, are on imprinted genes, particularly on those implicated in other neurodevelopmental disorders such as autism. Improper decoding of mCpG imprint marks would result in changes in gene expression patterns, resulting in the RS phenotype. The proximal region of chromosome 15 contains a large domain of imprinted genes involved in brain development and function including the AS gene, *UBE3A*, and is a strong candidate region for containing a maternally expressed autism gene. We have previously characterized *ATP10C*, which maps adjacent to *UBE3A*, and also demonstrates imprinted, maternal expression in normal human brain. However, preliminary examination using semi-quantitative RT-PCR of parental specific *ATP10C* expression in fibroblast cell lines from RS patients suggests that the ratio of maternal:paternal expression differs between clonal lines expressing wild-type or mutant *MeCP2* protein. Furthermore, levels of *ATP10C* are considerably reduced in a RS lymphoblast (LB) cell line expressing mutant *MeCP2*. This residual expression is of paternal origin, as is the majority of expression detectable from an LB line carrying an AS-imprinting center (IC) mutation, suggesting that both AS-IC and *MeCP2* mutations result in loss of maternal *ATP10C* expression. Preliminary results also demonstrate that a paternally expressed antisense transcript overlapping *ATP10C* is present in normal LBs, and that loss of maternal *ATP10C* expression in LBs with AS-IC and *MeCP2* mutations is coordinate with a gain of expression of an additional, maternal *ATP10C* antisense transcript in these cells. Together, these results suggest that *MeCP2* mediates control of the maternally expressed genes on 15q11-13 through the AS-IC, and that loss of expression of these genes may contribute to chromosome 15-associated autism and the AS and RS phenotypes. This work is supported by the NIH, CAN and IRSA.

**Identification of two highly conserved protein coding genes from the Prader-Willi syndrome candidate region.** *Y-H. Jiang*<sup>1</sup>, *H. Li*<sup>1</sup>, *L.G. Shaffer*<sup>1</sup>, *J. Lehoczky*<sup>2</sup>, *A.L. Beaudet*<sup>1</sup>. 1) Dept. of Molecular & Human Genetics, Baylor College Medicine, Houston, TX; 2) Center for Genome Research, MIT, MA.

Prader-Willi syndrome (PWS) is a neurological disorder characterized by neonatal hypotonia, childhood obesity, hyperphagia, and hypogonadism. Most PWS patients have either a paternal derived 4 Mb deletion of 15q11-q13 or maternal UPD. There are two common centromeric deletion breakpoints, the distal breakpoint (Class II) lies between D15S541/S542 and D15S543; and central breakpoint (Class I) being centromeric to D15S541/D15S542. A 2 Mb region from central breakpoint to *IPW* was defined as PWS candidate region, and paternally expressed transcripts (*SNRPN*, *PAR-1*, *PAR-5*, *IPW*, *ZNF127*, *NDN*, *MAGEL2*, *snoRNA*) were identified from this candidate region. However, none of these genes are yet proven to be the gene responsible for the PWS.

To identify additional transcripts from PWS candidate region, a sequence-based BAC contigs from which draft sequences have been deposited by human genome project were constructed and several novel transcripts were identified. Two protein coding genes, *PWSR-1* and *PWSR-2*, are mapped close to the centromeric breakpoint. *PWSR-1* has 30 exons and *PWSR-2* has 3 exons. *PWSR-1* is deleted in PWS patients with Class I deletion and the class II deletion may lie within the BAC containing the *PWSR-1* gene. Biallelic expression for both genes is detected in lymphoblasts with either class I or class II deletion by RT-PCR and northern. The mouse homologues of both genes were identified and show 98% and 96% identity to human at amino acid level. The *PWSR-1* protein is highly expressed in CNS and homologous to a p53 inducible protein. Both genes are highly conserved in many different species including *Drosophila*, *C.elegans*. A CpG island is identified in BAC containing *PWSR-1* gene by sequence analysis and examination of methylation status and tissue-specific imprinting are ongoing. Targeting vector for *PWSR-1* gene was constructed and inactivation of this gene in mouse ES cells is underway. Analysis of mice with a null mutation of *PWSR-1* may shed a light on the role of *PWSR-1* in PWS.

**Variation at the calpain 10 gene implicated in Type 2 diabetes susceptibility, plays no role in the pathogenesis of polycystic ovary syndrome.** *L. Haddad<sup>1</sup>, J. Evans<sup>2</sup>, S. Franks<sup>1</sup>, M.I. McCarthy<sup>1</sup>*. 1) Imperial College, London, UK; 2) Department of Diabetes and Vascular Medicine, University of Exeter, Exeter, UK.

Polycystic ovarian syndrome (PCOS) affects less than 5% of women, and shows clear physiological and epidemiological overlap with type 2 diabetes. Recent studies also indicate shared genetic susceptibility factors. The calpain 10 (CAPN10) gene was recently identified as underlying the major diabetes genetic effect localised to chr. 2q in Mexican Americans (MA). In this study, we applied family-based association methods in our PCOS resources to determine whether the CAPN10 gene plays a wider role in insulin-resistant states. We studied 146 parent-offspring trios (all European) ascertained through symptomatic, ultrasound-confirmed PCOS probands [median (IQR) age: 32.4 (25.6-39.2)y; BMI 24.0 (16.7-31.3)kgm<sup>-2</sup>]. A further 223 PCOS subjects were available for intermediate trait analyses. We analysed CAPN10 variants (SNPs 43, 44, 19, 63) implicated in diabetes-susceptibility, using PCR-based assays and the transmission disequilibrium test (TDT). Homozygosity for the common allele at SNP43 was most strongly associated with MA type 2 diabetes, but no association was evident in PCOS ( $c^2=0.08$ ). Furthermore, we observed no excess transmission at SNP44 ( $c^2=0.78$ ), SNP19 ( $c^2=0.59$ ) or SNP63 ( $c^2=4.51$ , nominal  $p=0.028$  (NS after correction)). Multilocus TDT (with TRANSMIT) revealed reduced haplotype diversity (only 5 of 16 possible haplotypes were present at  $>1.2\%$ ), but none showed excess transmission ( $c^2=7.68$ ,  $p=0.18$ ) to affected offspring. Haplotype frequencies in all 369 PCOS subjects did not differ from those in  $>800$  European control chromosomes ( $p=0.57$ ). Finally, intermediate trait analysis (for BMI, WHR, lipids, fasting insulin/glucose) revealed no relationship with genotype in all 369 PCOS subjects. We conclude that there is no association between CAPN10 variation and PCOS in European subjects, and, therefore, no evidence that CAPN10 plays a general role in susceptibility to insulin-resistant states.

**Inheritance of specific haplotypes of the IL4R gene associated with type 1 diabetes in the HBDI families.** *D.B.*

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Over 50% of the genetic predisposition to type 1 diabetes, a multifactorial autoimmune disorder, has been attributed to the HLA region, where multiple loci (e.g., HLA-DRB1, -DQA1, -DQB1, -DPB1 and -A), contribute to the genetic risk. It has proved rather difficult to identify specific susceptibility genes outside of HLA, despite the discovery of linked regions. We have used 282 multiplex type 1 diabetes families from the Human Biological Data Interchange (HBDI) to perform analyses of linkage and association to disease. We examined the transmission of alleles of candidate genes by genotyping SNPs in cytokine and cytokine receptor genes, using a multiplex PCR and immobilized probe array hybridization system.

SNPs in the IL4R gene, encoding a subunit of the interleukin-4 receptor, have been shown to be associated with Th2-type inflammatory diseases such as atopic asthma. We have genotyped the HBDI cohort at eight SNPs within the IL4R coding region: I50V, N142N, E375A, L389L, C406R, S478P, Q551R, and S761P. As these SNPs are genetically very close and there is strong LD among them, we could infer the transmission of eight-locus haplotypes in families. We performed molecular haplotyping for a subset of the SNPs as necessary to resolve linkage phase, using allele-specific PCR and immobilized probe hybridization. The association of IL4R with type 1 diabetes was examined using the Transmission-Disequilibrium Test (TDT) method. TDT analysis of individual loci indicates that the majority allele of the E375A, L389L, C406R and S478P SNPs is transmitted significantly more than 50% ( $p < 0.05$ ) to affected children. Furthermore, TDT analysis of haplotypes shows a significant ( $p < 0.01$ ) under-transmission of a single haplotype. This one putatively "protective" haplotype is composed of the minor alleles of many of the eight IL4R SNPs, alleles that have been reported to be associated individually with Th2-mediated disease. We hypothesize that a risk factor for the Th1-mediated type 1 diabetes might be conferred by non-inheritance of Th2-associated IL4R alleles or haplotype(s).

**Mutation analysis and association studies of the neurofilament L, M and H genes in German Parkinson's disease patients.** *R. Krueger*<sup>1</sup>, *N. Rahner*<sup>2</sup>, *C. Fischer*<sup>3</sup>, *T. Schulte*<sup>4</sup>, *C. Holzmann*<sup>2</sup>, *J.T. Epplen*<sup>3</sup>, *L. Schoels*<sup>4</sup>, *O. Riess*<sup>2</sup>. 1) Neurology, University of Tuebingen, Tuebingen, Germany; 2) Medical Genetics, University of Rostock, Rostock, Germany; 3) Human Molecular Genetics, University of Bochum, Bochum, Germany; 4) Neurology, University of Bochum, Bochum, Germany.

Recent advances in the understanding of the genetic contribution to Parkinson's disease (PD) indicate an involvement of pathological protein aggregation and disturbed protein degradation:  $\alpha$ -synuclein (SNCA) and ubiquitin C-terminal hydrolase (UCHL1) are components of Lewy bodies (LB) in brains of PD patients and are mutated in some rare forms of familial PD. Neurofilaments are a major component of LB aggregations and are composed of three neuron-specific proteins with molecular weights of 61kDa (NF-L), 90kDa (NF-M) and 110kDa (NF-H). Therefore we performed a detailed mutation analysis of the coding regions of all three genes encoding subunits of neurofilament in a large sample of 328 German PD patients. Using single strand conformation polymorphism analysis followed by direct sequencing we found three silent DNA changes (G163A, C224T, C487T) in the NF-L gene in three unrelated patients. Concerning the NF-M gene we identified three amino acid (aa) substitutions in PD patients that were found at similar frequencies in controls: Ala475Thr, Gly697Arg and Pro725Gln. In one index patient of a family with autosomal-dominantly inherited PD we found an in-frame deletion leading to the loss of valine in position 830 of the NF-M gene. Although absent in 754 chromosomes of healthy controls, no cosegregation with the disease could be observed in our family. In the NF-H gene we identified three novel aa substitutions in index patients with familial PD: Ala314Val, Arg352Ser and Asn390Thr. Association studies in sporadic PD using polymorphisms of all three genes revealed no significant difference in the allelic and genotypic distribution between patients and controls. Thus our study provides no genetic evidence for an involvement of neurofilament in the pathogenesis of PD. However, since disease causing mutations are expected to be rare, further studies are required to determine the relevance of neurofilament in PD.

**Polymorphisms of the NOTCH4 gene and schizophrenia.** *T.A. Klempan<sup>1</sup>, J.M. Trakalo<sup>1</sup>, C.N. Pato<sup>2</sup>, M. Pato<sup>2</sup>, M.-H. Azevedo<sup>2</sup>, F.M. Macciardi<sup>1</sup>, J.L. Kennedy<sup>1</sup>.* 1) Neurogenetics, CAMH, Toronto, ON, Canada; 2) Department of Psychiatry, SUNY at Buffalo, NY.

The human NOTCH4 gene is a member of a family whose products are known to be involved in embryonic specification of neuronal cell fate in *Drosophila melanogaster*. The NOTCH4 locus lies between the class II and class III regions of the major histocompatibility complex at 6p21.3, a region previously seen to be associated with schizophrenia. A recent report has demonstrated a highly significant association of 3 different polymorphisms across the promoter region and first exon of NOTCH4 with schizophrenia in trios of British descent. We have examined the transmission of the two most significant polymorphisms (CTG repeat and MspI SNP) in a combined sample of 60 mixed Caucasian and 96 Portuguese families with schizophrenia. TDT-sTDT and GENEHUNTER were used to study transmission of alleles and haplotypes to affected offspring. While MspI alleles were transmitted randomly to affected individuals ( $p=0.626$ ), several CTG repeat alleles demonstrated biased, though non-significant, transmission (CTG-10,  $p=0.123$ ; CTG-11,  $p=0.117$ ). Of the ten haplotypes examined, two showed significantly biased transmission (CTG-10/2,  $p=0.029$ ; CTG-11/2,  $p=0.033$ ). Considering the previous positive report, our results lend support for a possible susceptibility locus in the region of NOTCH4. We are currently examining an expanded sample and a third polymorphism.



**Identification of Disease Gene Variants Based upon Gene-Gene Interactions.** *G.P. Larson, L. Geller, T. Krontiris.*  
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New methods are needed for the identification of pathogenic alleles of candidate genes that may increase cancer susceptibility. Such risk alleles are expected to be of low penetrance and may act alone or modify the effects of other genes. We describe a simple, efficient strategy for detecting relatively rare genetic variants associated with human disease in small sample sizes. The method is based on (1) allele-sharing of candidate genes by affected siblings and (2) the postulation of gene-gene interactions. Candidate gene pairs are chosen based upon previous evidence demonstrating genetic or biochemical interaction. This approach, which we call Disease Association by Locus Stratification (DABLS), first stratifies ASPs based upon allele sharing of both alleles at a microsatellite marker linked to candidate 1. A second stratification is then performed based upon microsatellite marker sharing at candidate 2. Subsets of ASP sharing both alleles at both loci are identified as screening targets. Utilizing this approach in a breast cancer affected sib pair cohort, we have identified two non-coding disease haplotypes of *CDKN1A* by virtue of *BRCA1* and *TP53* interactions. Our results indicate that an approach based upon allele-sharing and gene-gene interactions will be valuable not only in identifying risk alleles, but also in elucidating their mechanism of action.

**Association studies of genetic risk factors for autistic disorder on chromosome 7.** *P.A. McCoy<sup>1</sup>, Y. Shao<sup>1</sup>, E.R. Martin<sup>1</sup>, G.R. DeLong<sup>1</sup>, M.L. Cuccaro<sup>2</sup>, J.R. Gilbert<sup>1</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Department of Medicine, Duke University Medical Ctr, Durham, NC; 2) W.S. Hall Psychiatric Institute, University of South Carolina, Columbia, SC.

Several genomic screens have indicated the presence of an autistic disorder (AutD) susceptibility locus within the distal long arm of Chromosome 7. Recent reports suggest two genes, WNT2 at 7q31 and RELN at 7q22, influence genetic risk in AutD. Both genes are also good functional candidates due to their involvement in development. WNT2 encodes a signaling protein that regulates cell growth and differentiation. RELN regulates neuronal migration during brain development. Using both the Pedigree Disequilibrium Test (PDT) and TRANSMIT (TMT), we tested for genetic association of two WNT2 variants in the 5'UTR and 3'UTR regions of WNT2 in 211 (multiplex and singleton) Duke AutD families. No significant association was found between AutD and the WNT2 genotypes. We also screened the coding region of WNT2 for two reported autism mutations (TG mutation in the signal domain of Exon 1 of WNT2 produced Leu5Arg and a CT mutation in Exon 5 produced Arg299Trp) and failed to identify these WNT2 mutations in our data set. Our data suggests that the WNT2 gene is not a major contributor to genetic risk in AutD.

In addition we examined the association of a 5'UTR polymorphism in RELN ((GCC)<sub>n</sub>) in a dataset (N=342 families) that included 252 Duke (multiplex and singleton) as well as 90 AGRE multiplex families. PDT and TMT analysis of the combined data found no evidence for association (p=0.12; p=0.09, respectively). However, examining the AGRE families independently resulted in support of association to RELN (p=0.0014 (PDT); p=0.02 (TMT)). Results in the overall Duke or subset by family type were not significant. Examining the allelic specific AGRE results showed association with the 8- and 10-repeat alleles. The previously reported AutD RELN association was with the longer triplet repeats (>10-repeat alleles). These data indicate that additional analysis of RELN is warranted in order to evaluate its role in AutD risk.

**Polysubstance abuse association genome scanning provides evidence for polygenic inheritance of common alleles at loci including BDNF and ADH.** *G.R. Uhl<sup>1,2</sup>, Q.-R. Liu<sup>1</sup>, D. Walther<sup>1</sup>*. 1) Molecular Neurology Branch, NIH/NIDA/IRP, Baltimore, MD; 2) Depts Neurol & Nsci, JHUSchool of Medicine.

Strong genetic contributions to drug abuse vulnerability are well documented, with much of this genetics overlapping with genetics for legal addictions. However, few chromosomal loci for substance abuse vulnerability alleles have been confirmed. We now identify chromosomal markers whose alleles distinguish drug abusers from controls in each of two samples, based on pooled sample SNP microarray and association analyses. Nine reproducibly-positive chromosomal regions defined by these markers and previous results from genome scans for ligal addictive substances were especially unlikely to have been identified by chance ( $P < 10^{-8}$ ). Positive markers identify the alcohol dehydrogenase (ADH) locus, the brain derived neurotrophic factor (BDNF) locus and seven other regions previously linked to nicotine or alcohol vulnerability. These data support polygenic contributions of specific common allelic variants to polysubstance abuse vulnerability.

**SLEB2 region in human SLE: locus-gene-mutation.** *L. Prokunina*<sup>1</sup>, *C. Castillejo-Lpez*<sup>2</sup>, *I. Gunnarsson*<sup>3</sup>, *L. Berg*<sup>4</sup>, *D. Tentler*<sup>1</sup>, *F. Öberg*<sup>1</sup>, *M. Alarcon-Riquelme*<sup>1</sup>. 1) Medical Genetics, Rudbeck Lab, Genetics and Pathology, Uppsala, Sweden; 2) Dept.of Cell and Molecular Biology, Lund University, BMC B13, 22184, Lund, Sweden; 3) Unit of Rheumatology, Karolinska Hospital,17176, Stockholm,Sweden; 4) MTC, Karolinska Institute,17176, Stockholm, Sweden.

In order to define the genetic component for Systemic Lupus Erythematosus (SLE), a complex autoimmune disease, we ran a genome scan for multicase families of Nordic origin (Iceland, Sweden and Norway). The locus SLEB2 was identified (Lindqvist, et al. *J Autoimm.* 14:169,2000). Further fine mapping has confirmed this locus and we have obtained a maximum multipoint Lod Score of 6.03 for markers D2S2585/D2S2985 (Magnusson et al. *Genomics*, 70:307, 2000). We have now used 8 SNPs in this region to construct haplotypes. We found a major haplotype segregating in multicase families (TDT test,  $p=0.00008$ ). We also analyzed 200 Swedish single case families and found the same major haplotype as in multicase families associated with SLE (TDT test  $p=0.00001$ ). Recombination analysis allowed us to significantly delimit the SLEB2 region. A candidate gene from the region is studied now. A functional SNP, associated with the disease is analyzed here.

**TGFA is a genetic modifier of Van der Woude Syndrome.** *T.M. Zuccherro, B.C. Schutte, S. Daack-Hirsch, J.C. Murray.* Dept Pediatrics, Univ Iowa, Iowa City, IA.

Van der Woude syndrome (VWS) is a common form of syndromic cleft lip and palate, accounting for approximately 2% of total cleft lip and palate cases. It is transmitted in an autosomal dominant pattern with at least 95% penetrance. Characteristics of VWS include cleft lip with and without cleft palate, isolated cleft palate, lower lip pits, hypodontia, and normal intelligence. Although a single gene locus for VWS has been mapped to 1q32-q41, occurrences of different forms of orofacial clefts within families and a previous report of a modifier locus on chromosome 17 (Sertie et al, 1999) suggest that this locus alone is not responsible for the manifestation of VWS. The presence of particular alleles at other loci may modify the severity of VWS in individual patients. Three excellent candidate genes are TGFA, MSX1, and TGFB3; these genes have been associated with nonsyndromic clefting in humans and TGFB3 and MSX1 have been shown to cause clefts in mouse knockout strains. We collected samples from 949 individuals in 129 families that have at least one member affected with VWS. To date, samples from both affected and unaffected individuals have been genotyped for a single polymorphism at each of the three candidate loci; Chi-square analysis was utilized to determine if particular alleles at these loci are associated with a particular cleft phenotype in our VWS population. No association was found at the MSX1 and TGFB3 loci. We found a significant association between an allele from the TGFA gene with the absence of lip pits in affected individuals ( $p=0.0007$ ). A significant association is also seen when compared to an unaffected internal family control population ( $p=0.01$ ) and an unaffected, unrelated control population (0.01). The results from an independent TDT (Transmission Disequilibrium Test) analysis are consistent with a role for the TGFA gene in modifying the lip pit phenotype ( $p=0.001$ ). Future studies will include exploring the mechanism for this association and genotyping additional alleles at these and other candidate loci.

**Mutations in the dystrophin gene are associated with sporadic dilated cardiomyopathy.** *J. Yan*<sup>1</sup>, *J. Feng*<sup>1</sup>, *C.H. Buzin*<sup>1</sup>, *J.A. Towbin*<sup>\*2</sup>, *S.S. Sommer*<sup>\*1</sup>. 1) Department of Molecular Genetics, City of Hope Medical Center, Duarte, CA; 2) Department of Pediatrics (Cardiology), Baylor College of Medicine, Houston, TX. \*These authors contributed equally to the work.

Dilated cardiomyopathy is the major indication for heart transplantation. Approximately 30% of all DCM is thought to be inherited, while 70% is sporadic. Mutations in the dystrophin gene have been associated with the uncommon X-linked form of dilated cardiomyopathy. We hypothesized that missense mutations and other less severe mutations might predispose to sporadic dilated cardiomyopathy. To test this hypothesis, we examined 22 patients with sporadic dilated cardiomyopathy by DOVAM-S (Detection of Virtually All Mutations-SSCP) (Liu et al, 1999. *BioTechniques* 26:932-942; Buzin et al, 2000. *BioTechniques* 28:746-753), a form of SSCP in which there is sufficient redundancy to detect virtually all mutations. Twenty-two kb of genomic dystrophin DNA was scanned in the 22 patients with sporadic DCM, including all 79 coding sequences and splice junctions, as well as six alternative exon 1 dystrophin isoforms. Three putative new mutations (IVS5+1 G>T, N2299T and F3228L) and six polymorphisms were identified. The splice site mutation IVS5+1 is predicted to cause skipping of exon 5, a region containing an actin binding site. In the missense mutation F3228L in exon 67, the amino acid phenylalanine is highly conserved in dog, mouse, chicken, Torpedo, dogfish, starfish, scallop, and amphioxus dystrophins. Screening of 141 control individuals failed to identify these 3 mutations, hinting that milder mutations in the dystrophin gene are associated with sporadic DCM, but clinical manifestation may require some environmental effector or a modifying gene. These mutations may have reduced penetrance and generally appear as *sporadic* DCM. Future studies are needed to confirm that mutations in the dystrophin gene are a frequent cause of DCM and to analyze families with these dystrophin mutations to search for environmental or genetic modifiers that may help to generate the disease phenotype.

**Fine mapping genes for Fc gamma receptor II and III in systemic lupus erythematosus.** C.A. Robertson, B.J. Morley, T.J. Vyse. Imperial College School of Medicine, Rheumatology Department, Hammersmith Campus, London, UK.

**Background** Human systemic lupus erythematosus (SLE) is a clinically heterogeneous autoimmune disorder, affecting primarily women. Autoreactive IgG antinuclear autoantibodies are a hallmark of the disease, and *in situ* formation of IgG immune complexes (IC) contributes to lupus glomerulonephritis. SLE is inherited as a complex trait, although the specific genes involved are largely unknown. Human genome-wide linkage analyses have mapped loci linked to SLE; several are orthologous with loci linked in murine models. One region, 1q23-24, is orthologous to murine susceptibility loci *Sle1*, *Lbw7*, *Nba2*, *Bxs3*. Candidate genes in this region include human IgG Fc gamma class II (*FCGR2A*, *2B*, *2C*) and III receptors (*FCGR3A*, *3B*). The receptors bind polymeric IgG and play a role in IC clearance and B cell signalling. A well-characterised polymorphism (H/R131) in *FCGR2A* influences receptor affinity: the low-binding allele (R131) is associated with SLE in some case control studies but not in others. It remains unclear if this polymorphism represents a true susceptibility allele.

**Methods** We have performed a family-based association study using a collection of 455 nuclear families of predominantly European Caucasian descent (86.8%). Genotyping of the *FCGR2A* H/R131 polymorphism was done with ARMS-PCR. We sequenced the promoter of *FCGR3B* and identified a novel polymorphism (-213G>T) which was typed using PCR/RFLP. Allelic association with SLE was examined by transmission disequilibrium testing (TDT) using TRANSMIT v3.5.

**Results** Transmission of *FCGR2A* H/R131 alleles did not vary significantly from expected values (families  $F=391$ ,  $n$  individuals=1897,  $P=0.267$ ); this was true regardless of renal status. No distortion in the transmission of *FCGR3B* -213G>T alleles was found among patients ( $F=274$ ,  $n=1396$ ,  $P=0.269$ ). It is unlikely that these polymorphisms represent SLE susceptibility alleles in European Caucasians.

We are currently genotyping additional polymorphisms at the *FCGR* locus in our family cohort and plan to define and analyse haplotypes.

**Evaluating resistin as a susceptibility gene for type 2 diabetes in the Finnish population.** *K. Silander*<sup>1</sup>, *C.M. Steppan*<sup>2</sup>, *K.L. Mohlke*<sup>1</sup>, *K.N. Lazaridis*<sup>1</sup>, *T.T. Valle*<sup>3</sup>, *T.A. Buchanan*<sup>4</sup>, *R.M. Watanabe*<sup>4</sup>, *M. Boehnke*<sup>5</sup>, *J. Tuomilehto*<sup>3</sup>, *R.N. Bergman*<sup>4</sup>, *M.A. Lazar*<sup>2</sup>, *F.S. Collins*<sup>1</sup>. 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) Dept. of Medicine and Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA; 3) Dept. of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; 4) Dept. of Medicine, Dept. of Preventive Medicine, and Dept. of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, CA; 5) Dept. of Biostatistics, University of Michigan, Ann Arbor, MI.

Resistin, a hormone secreted by adipocytes, has been suggested to play a role in insulin resistance in mice (Steppan et al. 2001, *Nature* 409:307-312). The Finland-United States Investigation of NIDDM genetics (FUSION) study aims to identify genetic factors contributing to type 2 diabetes. To evaluate whether genetic variants in human resistin are associated with type 2 diabetes we screened a cohort of 64 unrelated Finnish patients for possible gain-of-function variants. We also assessed possible association with insulin sensitivity by screening a cohort of 35 obese insulin-sensitive individuals for loss-of-function variants. These individuals were selected from a sample of approximately 600 unaffected individuals based on the criteria of having a BMI > 30 kg/m<sup>2</sup> and a minimal model insulin sensitivity index > 0.53 min<sup>-1</sup>/pM. The controls were 14 normoglycemic individuals at least 70 years old. The exons and promoter region of the human resistin gene were screened for variants using dHPLC followed by sequencing. Several polymorphic sites were identified in the promoter region, 5'-untranslated region, intron 3 and 3'-untranslated region. However, we did not detect any coding region variations either in diabetic patients or in any of the other subjects screened. Although no association was apparent, we are currently typing a larger set of control individuals for the polymorphisms identified to determine whether the allele frequencies differ among type 2 diabetics, obese insulin-sensitive individuals and elderly controls.



**Splice variants of small conductance calcium-activated potassium channel gene *KCNN3/ SKCa3* cause dominant-negative suppression of SKCa currents.** *H. Tomita*<sup>1,2</sup>, *V. Shakkottai*<sup>1</sup>, *H. Wulff*<sup>1</sup>, *G. Sun*<sup>1</sup>, *S.G. Potkin*<sup>2</sup>, *W.E. Bunney*<sup>2</sup>, *K.G. Chandy*<sup>1</sup>, *J.J. Gargus*<sup>1,3</sup>. 1) Dept Physiology and Biophysics, Univ California, Irvine, Irvine, CA; 2) Dept Psychiatry and Human Behavior, Univ California, Irvine, Irvine, CA; 3) Division Human Genetics, Dept Pediatrics, Univ California, Irvine, Irvine, CA.

*KCNN3/ SKCa3* is a member of the small conductance calcium-activated potassium channels that mediate slow afterhyperpolarizations following the action potential in vertebrate neurons. In the brain, *SKCa3* is most abundantly expressed in the substantia nigra and the ventral tegmental area, sites giving rise to two of the major dopaminergic pathways, and it regulates the spontaneous firing pattern of midbrain dopaminergic neurons. Recently, a 4bp deletion in *SKCa3* was identified in a patient with schizophrenia. This rare heterozygous deletion caused a frame-shift mutation that results in a truncated protein encompassing the entire N-terminus and lacking the hydrophobic core and C-terminus. This mutant protein has been shown to suppress SK channel function in a dominant-negative fashion, which would be expected to enhance the release of dopamine in the brain.

Herein we report identification of two novel splice variants of *SKCa3*, which we refer to as *SKCa3-b* and *SKCa3-c*, both lacking the N-terminus and the first transmembrane segment. *SKCa3-b* is expressed in brain and muscle along with the native *SKCa3-a* transcript, while *SKCa3-c* is present abundantly in muscle and lymphoid tissue. The *SKCa3-b* and *SKCa3-c* proteins are non-functional, but when expressed in PC12 cells produce dominant-negative suppression of endogenous SKCa currents by coassembling with native subunits into non-functional tetramers. The level of SKCa channel function may, therefore, be regulated by these splice variants. Taken together, these findings strongly suggest that brain region-specific expression of *SKCa3* splice variants may regulate dopaminergic activities and could potentially be related to the pathogenesis of schizophrenia.

**Molecular dissection of the promoter of the Human *KCNN3* / *SKCa3* gene.** *G. Sun*<sup>1</sup>, *H. Tomita*<sup>1,2</sup>, *V. Shakkottai*<sup>1</sup>, *J.J. Gargus*<sup>1,3</sup>. 1) Dept Physiology and Biophysics, Univ California, Irvine, Irvine, CA; 2) Dept Psychiatry and Human Behavior, Univ California, Irvine, Irvine, CA; 3) Division Human Genetics, Dept Pediatrics, Univ California, Irvine, Irvine, CA.

*KCNN3* /*SKCa3* is a member of the gene family, *KCNN1 - 4*, encoding the small and intermediate conductance calcium-activated potassium channels. Long CAG-repeat alleles of this gene have been found to be over-represented in patients with schizophrenia in a number of population-based association studies, and this large, recently sequenced 160 kb gene (AF336797) maps to human chromosome 1q21, a region recently implicated in schizophrenia by linkage.

In an attempt to determine the mechanisms by which the expression of this gene is regulated, we subcloned the genomic fragment covering 2,261 bp of the 5'-flanking region of *KCNN3* into a promoterless pGL3-luciferase vector, where it produced orientation-dependent expression of the reporter gene in transiently transfected PC12 cells, cells which natively express the channel. A series of 5'-deletion constructs of the promoter region were obtained and used in a transfection assay. The region between -325 bp to -529 bp upstream of the first codon contained the critical promoter region. Computer analysis of this 204 bp region predicted the presence of several transcription factor binding sites, including: NFY, SP1, NF1, NKX25, and two MZF1 sites. This region has a GC box and a CAAT box, but no TATA box. Primer extension analysis identified two transcription initiation sites. Site-directed mutations in the 204 bp minimal promoter region cloned into the promoterless pGL3-luciferase reporter vector were produced. Mutations altering the NFY motif caused a significant reduction in expression derived from the 204 bp region when constructs were used to transfect PC12 cells, suggesting that this motif plays a substantial role in *KCNN3* expression in these cells.

**The identification of gene expression signatures in the PBMCs of patients with Systemic Lupus Erythematosus.**

*E.C. Baechler*<sup>1</sup>, *P.M. Gaffney*<sup>1</sup>, *W.A. Ortmann*<sup>1</sup>, *F.M. Batliwalla*<sup>2</sup>, *G. Karypis*<sup>3</sup>, *P.K. Gregersen*<sup>2</sup>, *T.W. Behrens*<sup>1</sup>. 1) Medicine, University of Minnesota, Minneapolis, MN; 2) Human Genetics, North Shore University Hospital, Manhasset, NY; 3) Computer Science, University of Minnesota, Minneapolis, MN.

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease with a strong genetic component. SLE is characterized by unpredictable flares of disease activity and significant patient heterogeneity in drug response, severity of disease, and clinical symptoms. Here, we report our experience using Affymetrix microarrays to identify peripheral blood mononuclear cell (PBMC) gene expression signatures specific for SLE. In a first experiment, mRNA was isolated from the PBMCs of 11 SLE patients (with low levels of disease activity) and 11 healthy, age- and sex-matched controls. RNA probes were generated and hybridized to Affymetrix U95A GeneChips, followed by data analyses. This experiment identified 516 genes that differed significantly in their expression between SLE patients and controls ( $p < 0.05$  by unpaired T test). Among these were genes involved in cytokine and immune-cell signaling, a number of interferon-inducible genes, genes involved in calcium signaling pathways, as well as genes regulating the ubiquitin pathway of protein degradation. Hierarchical clustering clearly differentiated the patient profiles from the controls, and also identified at least 2 subgroups of SLE patients. A second experiment used polyA+ mRNA isolated from purified B and T cell populations, both at rest and after *in vitro* activation. This experiment identified 170 genes differentially expressed in resting B cells, 421 in resting T cells, and 244 in stimulated T cells (all at  $p < 0.05$  level). Once again, cluster analyses completely segregated SLE patients from controls, and many of the same genes identified in the PBMC experiment were also identified here. These results demonstrate that PBMC gene expression analysis can be used to identify deregulated pathways in SLE, and highlight the potential to identify patient sub-groups at the level of gene expression.

**MTHFR C677T and A1298C gene mutations, homocysteine, folic acid and vitamin B12 plasma levels and thrombosis in patients with systemic lupus erythematosus.** *H. Arrieta<sup>1</sup>, M.A. López<sup>1</sup>, A. Torre<sup>2</sup>, V. Valles<sup>3</sup>, M. Cardiel<sup>2</sup>, O.M. Mutchinick<sup>1</sup>.* 1) Dept de Genética; 2) Inmunología Reumatología; 3) Diabetes, Inst Nac Ciencias Médicas y Nutrición Salvador Zubirán, México, D.F.

**Background:** Thrombosis is considered a leading cause of morbidity and death in systemic lupus erythematosus (SLE). It is well known that the homozygous TT for the MTHFR C677T mutation produces a thermolabile enzyme that affects folate metabolism, homocysteine (Hc) remethylation in presence of methionine synthase and vitamin B12. The above induces a mild hyperhomocysteinemia (Hcy) that is a risk factor for thrombosis. **Objective:** To study the association of TT and CC genotypes for the MTHFR C<sup>677</sup>T and A<sup>1298</sup>C mutations, plasma levels of Hc, FA and B12, and daily intake of FA, B2, B6 and B12 with the risk of thrombosis in SLE. **Design:** Matched case-control study.

**Participants:** 35 patients with SLE and thrombosis (cases) and 70 with SLE without thrombosis (controls). **Methods:** Identification of the mentioned mutations by PCR and restriction enzyme analysis, plasma levels of Hc, FA and B12 by ELISA and the FA, B2, B6 and B12 intake by a food frequency questionnaire. .

**Results:** The frequency of TT genotype in cases was 14.7% and 11.4% in controls. We found only 1 homozygous for the C mutant allele in controls and none in cases. Correlation was not observed between TT and CC genotypes and plasma levels of Hc, FA and B12. No statistical differences were observed in daily intake of FA, B2, B6 and B12 vitamins among cases and controls. However, it was observed that the number of subjects with plasma level of FA below the median was significantly higher in cases than controls ( $P=0.035$ ).

**Conclusions:** The prevalence of the TT genotype of the C677T mutation in both groups of patients was significantly lower than the observed in diverse samples of normal Mexican populations studied by us. Our findings suggest that homozygous for the C677T and A1298C MTHFR mutations, plasma levels of Hc and B12 and current daily intake of FA, B2, B6 and B12 are not associated risk factors for thrombosis in SLE patients. Differences in FA plasma level most probably represents a spurious association.

**Identification of genetic polymorphisms in thymus-specific serine protease related to disease association with type 1 diabetes.** *B.A. Lie<sup>1</sup>, S. Johansson<sup>1</sup>, C.L. Bowlus<sup>2</sup>, J.R. Gruen<sup>3</sup>, F. Pociot<sup>4</sup>, J. Nerup<sup>4</sup>, E. Thorsby<sup>1</sup>, D.E.*

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We have previously shown that there are additional genes in the HLA complex, besides the DR and DQ genes, involved in the development of type 1 diabetes by testing microsatellites spanning the extended HLA complex. The marker D6S2223, located in the extended HLA class I region, was strongly associated with type 1 diabetes when the haplotype was fixed for DR3-DQ2. The gene encoding thymus-specific serine protease (PRSS16) is located in this genetic region, where we believed PRSS16 to be the most likely candidate gene among the known genes. PRSS16 is exclusively expressed in thymus and has been suggested to be involved in positive selection of T cells in the thymus. Therefore, we screened the promoter, introns and exons of PRSS16 (11216 bp) by DHPLC and direct sequencing, and identified 22 (19 novel) polymorphisms. Three of the polymorphisms were located in exons, one SNP giving rise to the amino acid substitution, one silent SNP and a 15 bp deletion. To test for disease association in large data sets of type 1 diabetes families, we selected these polymorphisms together with seven others, based on their frequencies, patterns of linkage disequilibrium and their probability of being biologically functional. However, none of these polymorphisms showed significant association with type 1 diabetes with a strength comparable to that observed for D6S2223. Hence, it is unlikely that PRSS16 is involved in the development of type 1 diabetes.

**Imprinted expression from single copy *Snrpn* transgenes in mice.** *S.J. Chamberlain, C.I. Brannan.* Department of Molecular Genetics and Microbiology and the Center for Mammalian Genetics, University of Florida, Gainesville, FL.

The Prader-Willi syndrome (PWS) candidate genes as well as the Angelman syndrome (AS) candidate gene are regulated by an Imprinting Center (IC) located 5' and including the first exon of the *SNRPN* gene. The elements that are necessary for the IC have been defined by means of deletion mutations that have been mapped in humans and created in mice. We have sought to determine the minimal sequence sufficient to confer imprinting to a transgene. Previously, we have shown that an 85 kb murine *Snrpn*P1 clone, containing approximately 30 kb of 5' flanking DNA exhibited imprinted expression when present as a two copy transgene, but exhibited biparental expression as a single copy transgene. This suggests that this 85 kb P1 clone contains sequences able to confer imprinted expression, but not sufficient to in a copy-number independent manner. Therefore, we have sought to add more 5' flanking sequence to determine if we can obtain reliable single copy imprinting of a transgene. To obtain imprinted expression of a single copy transgene, we have isolated and injected three new BAC clones spanning the *Snrpn* locus. We have obtained several intact transgenic lines from each BAC on the FVB/NJ genetic background. We have transmitted the transgene maternally and paternally and prepared total brain RNA. We assayed expression of the transgene by Northern blot (avoids detection of the background, low level expression derived from the maternal FVB/NJ allele, which is only detectable by RT-PCR). We are able to distinguish expression of the transgene from expression of the endogenous gene by using a marked endogenous allele. We have found that single copy transgenes containing only 8 kb of upstream *Snrpn* sequence are expressed upon both maternal and paternal inheritance. In contrast, we found that single copy transgenes containing either 90 kb or 180 kb of upstream sequence are expressed upon paternal, but not maternal transmission. These data suggest that the relevant sequences for single copy imprinting lie within a 90 kb region upstream of *Snrpn*.

**Monozygotic twins discordant for the Beckwith-Wiedemann syndrome exhibit the same epigenetic defect at the 11p15 locus.** *C. Gicquel<sup>1</sup>, V. Gaston<sup>1</sup>, P. Sarda<sup>2</sup>, V. Soupre<sup>3</sup>, M.P. Vazquez<sup>3</sup>, Y. Le Bouc<sup>1</sup>.* 1) Laboratoire d'Endocrinologie - INSERM U515, Hopital Trousseau, AP-HP, Paris, France; 2) Service de Genetique Medicale et Foetopathologie, Hopital Arnaud de Villeneuve, Montpellier, France; 3) Service de Chirurgie Maxillo-faciale, Hopital Trousseau, AP-HP, Paris, France.

Monozygotic twinning occurs with relatively high frequency in Beckwith- Wiedemann syndrome (BWS). Most of monozygotic twins with BWS are female and in most cases the twins are discordant for the BWS phenotype. It has been suggested that the excess of female monozygotic twin pairs may be related to the process of X chromosome inactivation. We report here two pairs of female MZ twins, each discordant for BWS. In both cases, one twin showed typical BWS whereas the other did not. One pair of twins had a 47,XXX karyotype. The allelic status of the 11p15 region and the methylation status of the KCNQ1OT and H19 genes were investigated in DNA from peripheral blood cells by Southern-blot analysis. X chromosome inactivation was determined at the M27 locus (Xcen-p11.22) by Southern-blot analysis. Analysis of the 11p15 region showed the same epigenetic defect (almost complete demethylation of the KCNQ1OT gene) in both BWS twins and in normal twins. The X chromosome inactivation pattern was also similar in BWS patients and their unaffected twins. These data do not support an association between the expression of BWS and X chromosome inactivation. The hypothesis of a different tissue distribution of epimutated cells in the two twins is unlikely because of the complete demethylation of the KCNQ1OT gene and the absence of any clinical signs in the two unaffected twins. Further studies are necessary to establish the mechanism of discordant monozygotic twinning in BWS.

**Analysis of imprinting in the Silver Russell Syndrome candidate gene region at 7q31-qter.** *L. Bentley<sup>1</sup>, D. Monk<sup>1</sup>, E. Smith<sup>1</sup>, R. Edmonds<sup>1</sup>, M. Hitchins<sup>1</sup>, M.A. Preece<sup>2</sup>, P. Stanier<sup>1</sup>, G.E. Moore<sup>1</sup>.* 1) Maternal and Fetal Medicine, Imperial College, London, UK; 2) Institute of Child Health, University College London, London, UK.

Silver Russell syndrome (SRS) is characterised by pre- and postnatal growth restriction in association with other dysmorphic features including asymmetry and fifth finger clinodactyly. The syndrome is heterogenous, with a number of chromosomes implicated. However, maternal uniparental disomy for chromosome 7 (mat UPD7) has been demonstrated in up to 10% of cases. Two imprinted candidate regions on chromosome 7 have been identified by cytogenetic abnormalities on both the p and q arms. The first, defined by two unrelated maternally derived duplications of 7p11.2-p13 includes the reciprocally imprinted growth suppressing *GRB10* gene. Recently Hannula *et al.* (2001) reported a single case of segmental mat UPD7 of 7q31-qter. This defines the second candidate gene region that includes the imprinted gene cluster comprising of *MEST*, *COPG2* and *MIT1/LB9*.

Using 8 polymorphic repeat markers for the q arm, we systematically re-evaluated the possibility that segmental UPD7 may be present in 17 probands from our SRS cohort. In addition we have found that the *MEST* gene replicates asynchronously, a common feature of an imprinted gene domain. Using individual PAC and BAC clones from a contig of the region as FISH probes, we have delineated the extent of the asynchronous domain. The imprinting status of genes known to map within this region were assessed in monochromosomal somatic cell hybrids retaining individual chromosome 7s of known parental origin, in which the paternal expression of the *MEST 1* isoform was maintained.



**Microarray analysis of transcript expression in Prader-Willi syndrome.** *D.C. Bittel, Z. Talebizadeh, M.G. Butler.* Children's Mercy Hospital and University of Missouri-Kansas City School of Medicine, Kansas City, MO.

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, hypogonadism, hyperphagia, early onset of childhood obesity, a particular facial appearance and mental deficiency. PWS is caused by deficiency of genes located on the proximal long arm of chromosome 15. To date, several genes have been identified in the 15q11-q13 region including ZNF127, NECDIN, MAGEL2, SNURF-SNRPN, IPW, UBE3A, ATP10C, GABRA5, GABRB3, GABRG3, P and HERC2. ZNF127, NECDIN, MAGEL2, SNURF-SNRPN and IPW are expressed from the paternal chromosome and the lack of expression contributes to PWS. The other genes are either maternally expressed (UBE3A, ATP10C) or have biallelic expression. Deletion of paternal 15q11-13, maternal uniparental disomy (UPD) of chromosome 15 or in rare cases, imprinting center (IC) mutations are reported to cause PWS. The proximal portion of chromosome 15 which is critical for PWS contains approximately 4 million base pairs of DNA and may contain as many as 100 expressed sequences or genes. We have generated a custom cDNA microarray by mining the GenBank database for identification of PCR primer sequences from the proximal region of chromosome 15, performing PCR reactions and spotting the PCR products on glass slides. A total of 90 nonredundant genes and ESTs from the proximal long arm of chromosome 15 were spotted. Twenty- six of these have been mapped between the pTel and D15S122 anchor markers, 7 between D15S122 and D15S156, 10 between D15S156 and D15S165, 7 between D15S165 and D15S144, 12 between D15S144 and D15S118, 6 between D15S118 and D15S146, 8 between D15S146 and D15S117, 1 between D15S117 and D15S159 and the rest were either distal or not accurately mapped. Five micrograms of mRNA from actively growing control and PWS lymphoblastoid lines were labeled alternately with Cy3 (green) or Cy5 (red) and pooled for hybridization to the array. We compared and analyzed the expression of sequences from the proximal 15q region between control lymphoblasts and lymphoblasts derived from individuals with PWS caused by deletions, UPD and IC mutations.

**NTera2 as a Cell Culture Model for Brain-Specific Imprinting on Chromosome 15q11-q13.** C.J. GIULIANO, M. LALANDE. DEPARTMENT OF GENETICS AND DEVELOPMENTAL BIOLOGY, UNIVERSITY OF CONNECTICUT HEALTH CENTER FARMINGTON, CT.

Prader-Willi Syndrome (PWS) results from a physical or functional loss of paternally expressed genes in the chromosome 15q11-q13 region. Angelman syndrome (AS) results from mutation or absence of a maternally expressed gene, UBE3A. Genomic imprinting within the PWS/AS region is believed to be coordinately regulated by a bipartite imprinting center, implying that genomic imprinting is under regional control. There are differences in the regional distribution of non brain-specific and brain-specific transcripts in the PWS/AS region (Lee & Wevrick, 2000). Genes with broader tissue expression patterns are located in the more centromeric 15q11-q13 region; these include, ZNF127, NECDIN, MAGEL2, SNRPN, PAR5, PAR7, IPW, PAR1, and small nucleolar (sno) RNAs HBII85 and HBII13. In contrast, transcripts that show brain-specific expression are located in the more telomeric region; these include, UBE3A antisense (UBE3A-ATS), A005C48, and tandem repeats of the HBII52 snoRNAs. A complex pattern of alternative splicing is observed for the brain-specific genes, and these may be processed from one large transcript. The NTera 2 (NT2) cell line will be used as a model system to study the organization of the two expression domains. NT2 cells can be induced to differentiate into neurons by treatment with retinoic acid (RA). Expression of IPW, PAR5 and the HBII85 snoRNAs is observed throughout the process of neuronal differentiation, including in undifferentiated NT2 cells. This expression pattern is consistent with the presence of IPW, PAR5 and HBII85 in the domain with broader tissue expression. In contrast, expression of A005C48 and the HBII52 snoRNAs is not detected until 3 weeks after induction of neuronal differentiation, with high levels of expression evident in mature neurons. This pattern of expression is consistent with the localization of A005C48 and the HBII52 snoRNAs within the brain-specific domain. The *in vitro* system of neuronal NT2 cell differentiation may provide a powerful tool for studying the mechanism of brain-specific imprinting in the PWS/AS region. Reference: Lee, S. and Wevrick, R. (2000) American Journal of Human Genetics, 66: 848-858.

**Genetic mechanisms connecting disturbed early growth and type 2 diabetes: further evidence for imprinting at the insulin gene locus.** *N. Gharani*<sup>1</sup>, *S. Huxtable*<sup>1</sup>, *L.K. Li*<sup>2</sup>, *S. Humphries*<sup>2</sup>, *P. Hindmarsh*<sup>2</sup>, *M. McCarthy*<sup>1</sup>. 1) Imperial College; 2) UCL, London, UK.

There is growing evidence that the association between early growth and metabolic phenotypes in adults, is in part, due to the pleiotropic effects of genes modulating insulin secretion and/or action, and thereby contributing to variation in both fetal and adult phenotypes. One such locus is the insulin gene. Recent studies have shown that *INS*-VNTR-associated susceptibility to type 2 diabetes is preferentially-transmitted through paternal class III alleles, and is, by implication, mediated through imprinted mechanisms active in early life. We reasoned that similar imprinting effects modulating the known association between *INS*-VNTR class and early growth would provide further support for a common genetic mechanism. Therefore, we examined *INS*-VNTR genotypes of 191 European parent-offspring trios, each ascertained through unselected consecutive births. Measurements of infant weight, length and head circumference were available at 0, 6 and 12 months of age. Parental and cord-blood samples were genotyped for the -23Hph1 variant (as a surrogate for VNTR class), with ambiguities in the parental origin of fetal alleles resolved through additional *INS*-VNTR class I typing. The search for imprinting effects focussed on the 51 heterozygous infants, 21 of whom had inherited a paternal class I, and 30 a paternal class III. No differences were seen at birth, but, by 6 months, there was a clear divergence in growth patterns, with the paternal class III babies both lighter (weight SDS (SD): 0.03(1.02) vs 0.66(0.90),  $p=0.029$ ) and shorter (length SDS (SD): 0.13(1.00) vs 0.90(0.81),  $p=0.006$ ) than those with paternal class I. In this study, we found no evidence for a relationship between fetal VNTR genotype (I/I vs I/III vs III/III,  $p>0.32$ ) and parameters at birth, in contrast with previous ALSPAC data (though numbers were smaller). These data provide the first evidence that VNTR-associated effects on early growth, like those on diabetes, demonstrate parent-of-origin effects, lending additional support to a genetic contribution to the link between fetal and adult phenotypes.

**Identification and Characterization of Imprinting on Human Chromosome 14.** *C.J. Clarke, D.A. McFadyen, J. Shah, D.W. Cox.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Genomic imprinting is known to significantly influence development, growth, and behavior in mammals. Imprinting has been directly linked to the etiology of several human disorders. Patients with both paternal and maternal uniparental disomy (UPD) for chromosome 14 have distinct phenotypes which have raised interest in possible imprinted regions on human chromosome 14.

To aid in the understanding of the phenotypes of UPD14 and 14q deletion patients we have performed an analysis using cell lines collected from patients with maternal UPD, paternal UPD or a deletion in 14q32. Using an RT-PCR based assay, we determined the imprinting status of 28 known genes and 67 ESTs located throughout human 14q32. This chromosomal region contains two previously identified reciprocally imprinted genes *GTL2* and *DLK1* and four associated imprinted transcripts *DAT*, *PEG11*, *antiPEG11*, *MEG8*. In our study, three novel paternally expressed transcripts were also identified, however, the function of the newly identified imprinted transcripts is unknown. This work provides the framework for further investigation into the function of these transcripts, their role in determining the phenotype in UPD14 patients, as well as the identification of additional imprinted genes in 14q.

**The human *LIT1* CpG island is a putative imprinting control element playing an essential role in Beckwith-Wiedemann syndrome.** *S. Horike, K. Mitsuya, M. Meguro, N. Kotobuki, A. Kashiwagi, T. Notsu, F. Ayabe, Y. Shirayoshi, M. Oshimura.* Department of Molecular & Cell Genetics, School of Life Science, Faculty of Medicine, Tottori University, Yonago, Tottori, Japan.

Imprinting defects at the human chromosome 11p15.5 lead to Beckwith-Wiedemann syndrome (BWS). The molecular mechanisms underlying genomic imprinting are unknown, but it is of particular interest in the context of coordinated regulation of neighbouring genes on the megabase scale. Two imprinting control elements (IC) that guide the allelic expression of neighbouring genes in the imprinting cluster, have been localized to the *H19/IGF2* locus and *KvLQT1/LIT1* locus in the human chromosome 11p15.5. It is thought that the imprinting of *H19/IGF2* locus is dependent upon an IC that acts as a chromatin insulator to interfere with enhancer-promoter interaction. We have previously shown that targeted deletion of a putative IC within the *KvLQT1/LIT1* locus abolished the *LIT1* transcription on the paternal allele, accompanied by activation of the multiple normally suppressed paternal alleles including *KvLQT1* and *p57<sup>KIP2</sup>*. We also have suggested that the IC within the *KvLQT1/LIT1* locus could have boundary functions in addition to being a promoter region for the *LIT1*, and this could be also true for the IC within the *H19/IGF2* as well. The similarities in the imprinting characteristics of these two ICs suggest that the imprinting element would be conserved in the heterologous chromosomal locations within one species, as well as in the orthologous regions between species. To test this hypothesis, we generated the modified chromosome harboring the *LIT1* locus, in that the *H19/Igf2* IC was inserted using chicken DT40 cells. Analysis of expression status of neighbouring genes in the *LIT1* locus using the modified chromosome is now ongoing. This work was supported by CREST of Japan Science and Technology Corporation (JST), and grants from the Mitsubishi Foundation, the Ministry of Health and Welfare of Japan and the Human Frontier Science Program Organization (HFSPO).

**Brain-specific processing of an imprinted transcript encompassing *Ipw* and the *Ube3a* antisense.** *M. Landers*<sup>1</sup>, *D. Bancescu*<sup>1</sup>, *C. Rougeulle*<sup>2</sup>, *H. Glatt-Deeley*<sup>1</sup>, *M. Lalande*<sup>1</sup>. 1) Genetics, UCHC, Farmington, CT; 2) Institut Pasteur, France.

Angelman syndrome is caused by the absence of a maternal contribution to the 15q11-q13 region. Most cases are due to a deficiency of *UBE3A/E6AP*, a gene with maternal-specific expression in brain. We have recently identified a paternal-specific transcript, *UBE3A-ATS*, that is also brain-specific and is antisense to *UBE3A*. Our hypothesis is that the *UBE3A-ATS* silences the paternal allele of *UBE3A* in brain although the mechanism of this interaction remains unknown. We characterized the upstream region of the mouse ortholog, *Ube3a-ATS*, in order to understand the regulation of its transcription in mouse brain. Strand-specific RT-PCRs were performed starting from the 3' region of *Ube3a*. Comparison of the nucleotide sequence of the genomic DNA and the RT-PCR products revealed that the ATS is contiguous with genomic DNA extending to 10 kb downstream of the *Ube3a* 3'-end. We detected two alternatively spliced ATS transcripts and identified eight exons spanning approximately 35 kb of genomic DNA further downstream. Three of these exons showed 82% homology to the exon G of the *Ipw* (*Hum. Mol. Genet.* 6:325) transcript. The genomic sequence also revealed a copy of the MBII52 small nucleolar RNA (snoRNA) embedded between *Ipw* exons G2 and G1 for one of the ATS transcripts. MBII52 snoRNAs are paternally expressed, brain-specific and processed from introns of the *Ipw* transcript (*PNAS* 97:14311). Consistent with these data, that suggest that *Ipw* and the *Ube3a-ATS* are part of the same transcript, we PCR-amplified a fragment from *Ipw* exons B to E by priming an RT from the contiguous region of ATS that overlaps the *Ube3a* 3'-end. We then used the P19 cell line, an *in vitro* model for neuronal differentiation, to analyze the expression of the paternal brain transcripts. *Ipw*, MBII52 snoRNAs and *Ube3a-ATS* are first detected at day 7 after induction of P19 cell differentiation. The temporal coincidence of these three transcriptional events during neurogenesis, together with the finding that both *Ipw* and *Ube3a-ATS* are under the control of the PWS-IC (*Genomics* 73:316), indicate that processing of *Ipw/Ube3a-ATS* in brain may play an important role in regulating the imprinting of *Ube3a*.

***NDN and MAGEL2 as candidate genes in Prader-Willi Syndrome.*** *S. Lee, S.L. Kuny, C. Walker, R. Wevrick.* Dept Medical Genetics, Univ Alberta, Edmonton, AB, Canada.

Prader-Willi syndrome (PWS) is a contiguous gene syndrome caused by the loss of imprinted, paternally expressed genes from human chromosome 15q11-q13. PWS is a neurobehavioural disorder characterized by neonatal hypotonia, hyperphagia and developmental delay. *NDN* and *MAGEL2*, members of the MAGE gene superfamily, are two adjacent, paternally expressed genes that are silenced in PWS. The mouse orthologues, *Ndn* and *Magel2*, are located within a region of conserved synteny on mouse chromosome 7. We have examined *Ndn* and *Magel2* in mouse P19 embryonic carcinoma cell lines. P19 cells can be induced to differentiate into neurons with the addition of retinoic acid and are thus an *in vitro* model of neurogenesis. We have found that the expression of *Magel2* RNA is repressed in P19 cells with the addition of retinoic acid, whereas *Ndn* expression is induced. The complementary expression pattern of these genes suggests that they have distinct roles in neurodevelopment despite their sequence similarities. The expression of *Ndn* and *Magel2* by RNA *in situ* hybridization in developing mouse embryos also suggests complementary roles in early nervous system development. *Ndn* expression begins around embryonic stage E9 and is distributed throughout the nervous system. *Magel2* expression begins around day E11, and confined mainly to the central nervous system. We hypothesize that the combined loss of *NDN* and *MAGEL2* may contribute to the neurological problems associated with PWS.

**Inheritance patterns of alleles in imprinted regions of the mouse genome at different stages of development.** A.K. Naumova<sup>1,4</sup>, S. Croteau<sup>1</sup>, C.M.T. Greenwood<sup>2,4</sup>, K. Morgan<sup>3,4</sup>. 1) Department of Obstetrics and Gynecology, Women's Pavillon, F3.36, Royal Victoria Hosp, Montreal, Quebec, Canada; 2) Department of Epidemiology and Biostatistics, McGill University and Research Institute of the McGill University Health Centre; 3) Department of Medicine, McGill University and Research Institute of the McGill University Health Centre; 4) Department of Human Genetics, McGill University, Montreal, Quebec, Canada.

Deviations from Mendelian 1:1 transmission ratio have been observed in mice and humans. With few exceptions the mechanism leading to transmission-ratio distortion (TRD) remains obscure. We proposed that a genomic imprinting mechanism plays a key role in the genesis of grandparental-origin dependent TRD (Naumova et al. 2001). To further test this hypothesis, we analyzed the transmission of grandparental alleles at three imprinted regions of the mouse genome known to contain genes required for embryo development. We found and replicated moderate (58%: 42%) TRD in favor of grandmaternal alleles in the imprinted region of maternal distal chromosome 12 among female offspring (326 grandmaternal alleles versus 238 grandpaternal alleles inherited, exact binomial  $p = 0.0062$ ). The region of maximal distortion on chromosome 12 was mapped distal to position 50 cM, and includes the imprinted genes *Meg3* and *Dlk1*. Comparison of transmission ratios at the distorted region of chromosome 12 among 3-week old mice with those in 3.5 d.p.c. and 7.5 d.p.c. embryos suggests that the distortion in favor of grandmaternal alleles is due to postimplantation embryo loss. The absence of grandparental-origin dependent TRD for maternal chromosomes 6 and 7 suggests that the relationship between TRD and imprinting is complex. Most likely multiple conditions are required for TRD to occur.



**Complementary patterns of histone H3 Lys9 and Lys4 methylation at the Prader-Willi imprinting center. J.**

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Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are clinically distinct syndromes both caused by genetic alterations in the chromosome 15q11-q13 region. PWS is caused by lack of expression of paternally-expressed genes in this region; AS is caused by lack of a functional maternal allele of UBE3A, which is imprinted only in brain. A 4.3 kb region that includes the promoter and exon 1 of SNRPN, referred to as the PWS imprinting center (PWS-IC), is required for establishment of the paternal pattern of gene expression and DNA methylation throughout the PWS/AS region. This region shows cytosine methylation on the maternal allele in somatic cells. However, recent studies have shown that this DNA methylation is not present in human oocytes, but occurs after fertilization and therefore is not the gametic imprint (El-Maarri et al.(2001) Nature Genet. 27:341-4). We have analyzed other epigenetic modifications of this region in order to identify the gametic imprint. We have used chromatin immunoprecipitation to show that histone H3 methylated on Lys9, which has been associated with chromatin-based silencing in fission yeast and *Drosophila*, is associated preferentially with the silenced maternal promoter of SNRPN. Histone H3 methylated on Lys4 is associated preferentially with the active paternal promoters of SNRPN and NDN. In this region, histone H3 methylated on Lys9 and Lys4 correlate with alternative states of inactive and active chromatin, respectively. Methylation of histone H3 on Lys9 on the maternal allele of the PWS-IC is therefore a candidate gametic imprint for this region. Replacement of histone by protamine during spermatogenesis would provide a convenient means of erasing a maternal histone-based imprint.

**Role of DNA methylation and histone acetylation in silencing of imprinted genes in Prader-Willi syndrome.** K. Takano, M. Takahashi, A. Sudo, T. Wada, S. Saitoh. Department of Pediatrics, Hokkaido University School of Medicine, Sapporo, Japan.

Prader-Willi syndrome (PWS) is associated with silencing of several imprinted genes located in 15q11-q13. These genes are epigenetically silenced through genomic imprinting. We have previously demonstrated that the CpG island of *SNURF-SNRPN*, which is a key imprinted locus in 15q11-q13, is not only heavily methylated but is also hypoacetylated on the inactive maternally derived allele. We have extended the investigation of histone acetylation status to other imprinted loci including 4 paternally-expressed genes (*MKRN3*, *MAGEL2*, *NDN*, and *IPW*), and 2 maternally-expressed genes (*UBE3A*, and *ATP10C*). Lymphoblastoid cells from PWS and Angelman syndrome (AS) patients, and controls were subjected to a chromatin immunoprecipitation (ChIP) assay using anti-acetylated histone H3 and H4 antibodies. Parent-of-origin specific histone acetylation was not demonstrated in these loci except for at *SNURF-SNRPN*, even at the CpG island of genes where parent-of-origin specific DNA methylation had previously been demonstrated (ie., *MKRN3*, *MAGEL2*, *NDN*). The *UBE3A* promoter is biparentally unmethylated and was associated on both allele with hypoacetylated histones. The CpG island of *ATP10C* was also associated on both allele with hypoacetylated histones. To further elucidate the role of DNA methylation and histone acetylation in silencing of the imprinted genes, we treated the cells with 5-azadeoxycytidine (5-aza-dC), an inhibitor of DNA methylation, trichostatin A (TSA) that is a specific inhibitor of histone deacetylase, and a combination of both drugs. 5-aza-dC treatment induced expression of *SNURF-SNRPN* and *IPW*, but TSA alone failed to reactivate either gene consistently. A combination of 5-aza-dC and TSA did not further increase the expression level of either gene compared with 5-aza-dC alone, although it increased the histone acetylation level at the CpG island of *SNURF-SNRPN*. These results indicate that DNA methylation may play a major role in maintaining silencing of the imprinted genes in 15q11-q13.

**Molecular analysis of the cluster of imprinted gene on 11p15 in a cohort of BWS patients.** *S. Russo<sup>1</sup>, M. Mencarelli<sup>1</sup>, D. Milani<sup>2</sup>, F. Cavalleri<sup>1</sup>, A. Selicorni<sup>2</sup>, L. Larizza<sup>1,3</sup>.* 1) Molecular Biology, Institute Auxologico Italiano, Milan, Italy; 2) Genetic Department, I.C.P. Hospital, Milan, Italy; 3) Biology and Genetics Dept, University of Milan, Italy.

The Beckwith-Wiedemann syndrome (BWS) is the most common overgrowth syndrome mainly characterized by anterior abdominal wall defects, macroglossia and gigantism and associated with an increased susceptibility (7.5%) to a variety of childhood tumors including Wilms tumors, adrenocortical carcinoma and hepatoblastoma. Cytogenetic and molecular anomalies involving the 11p15.5 region, containing at least eight imprinted genes, point to this region, as the mainly involved in the aetiology of BWS. We collected a cohort of 26 patients affected by BWS: 11 of them filled all the major and minor clinical criteria of the syndrome, 9 had an intermediate phenotype and 6 showed only isolated hemihypertrophy, which is considered a very mild form of this syndrome. Among the 11 classical BWS 2 familial cases were identified. karyotype was normal in all the patients. Paternal segmental uniparental disomy within the of 11p15.5 region, investigated in all the patients by segregation of microsatellite markers, was found in 3 patients affected by classical BWS, in 1 with an intermediate phenotype and 1 with isolated hemihypertrophy. Analysis of methylation pattern of the imprinted gene H19 was found in a complete BWS patient, while mutation screening of the p57 coding region, performed by direct sequencing, did not evidence any alteration. IGF2 methylation pattern is in progress in all the patients. Our data confirm the incidence of 11pat UPD in 20% of BWS and point to this mechanism as responsible also for an incomplete clinical picture. Mutations of p57 gene and alteration of the H19 imprinting may be involved only in severe BWS form.

**Identification of parent-of-origin-specific *in vivo* footprints in the Prader-Willi syndrome imprinting center. S.**

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The Prader-Willi and Angelman Syndromes (PWS/AS) region is located on human chromosome 15 q11-q13 and includes a cluster of imprinted genes that are coordinately regulated. Microdeletions in patients with imprinting mutation have defined an imprinting center (IC) which appears to control resetting of imprinting in the germ line and postzygotic maintenance of imprinting throughout the region. The IC is located 5' to the *SNURF-SNRPN* gene and has a bipartite structure, one component being involved in PWS and the other in AS. We are interested in identifying *cis*-acting elements that may mediate the IC function. Therefore we have performed *in vivo* footprint analysis with dimethylsulfate (DMS) and DNase I on the promoter region of the *SNURF-SNRPN* gene on lymphoblasts derived from PWS and AS patients. This region is located within the PWS-IC and shows differential DNase I hypersensitivity, DNA methylation and histone acetylation between the paternally- and the maternally-inherited alleles. DMS *in vivo* footprinting has identified a series of sequence-specific DNA-protein interactions within 85 bp that flank the transcription initiation site. Most of these footprints are specific to the transcriptionally active paternally-inherited allele, however one footprint is specific to the maternally-inherited allele. DNase I *in vivo* footprinting has shown that part of *SNURF-SNRPN* exon 1 and adjacent intronic sequences are protected from enzymatic cleavage specifically on the paternally-inherited allele. Additionally we have identified two footprints specific on the maternally-inherited allele in and upstream of exon 1. One or more of these parent-of-origin-specific *in vivo* footprints are likely to reflect the location *cis*-acting regulatory elements that play a role on transcriptional activation and/or repression of the *SNURF-SNRPN* gene. In addition, they may also act as *cis*-acting elements associated with IC function.

**Duplication of the early developmental regulator 3 locus, EDR3, in Cornelia de Lange syndrome. A.M.**

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We have identified EDR3, a novel gene encoding member of the polycomb group (PcG) gene family. EDR3 maps to chromosome 3q26.2-q26.3, a region implicated in the pathogenesis of Cornelia de Lange syndrome (CDLS). This syndrome is typified by a characteristic facies with microbrachycephaly, synophrys, micrognathia and prominent philtrum as well as other abnormalities such as pre- and postnatal growth delay, mental retardation, hirsutism, delayed skeletal maturation and upper limb abnormalities. FISH and Southern analyses reveal that EDR3 is consistently duplicated in cell lines derived from CDLS patients, even in the absence of gross cytogenetic abnormalities. Analyses indicate that EDR3 is expressed during development in the craniofacial region, limbs, brain, heart and, liver. The gene has an ORF of 2895 bp and is organized into 16 exons. The EDR3 protein is similar to other members of the PcG family including human EDR1 and EDR2. Members of the PcG family regulate gene expression through protein-protein and protein-DNA interactions and play an important role in axial patterning of organisms through regulation of expression of homeotic genes. PcG protein function exhibits marked dosage sensitivity and alterations in gene copy number result in anterior or posterior transformations and developmental dysmorphogenesis. Based on the observed localization of the EDR3 gene, its consistent duplication in CDLS patients, and its expression pattern and potential role in development of tissues affected in CDLS, we propose that overexpression of EDR3 caused by increased gene copy number is responsible, at least in part, for the phenotype associated with CDLS.

**Transmission Disequilibrium Mapping in the Serotonin Transporter Gene (*SLC6A4*) Region in Autistic Disorder.**

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The serotonin transporter gene (*SLC6A4*) is a candidate gene in autistic disorder based on neurochemical and neuroendocrine studies and the efficacy of potent serotonin transporter inhibitors in reducing rituals and aggression. Two polymorphisms, 5-HTTLPR in the promoter region and a variable number of tandem repeat polymorphism (VNTR) in the second intron, were previously identified and suggested to modulate transcription. In the present study, we further investigated *SLC6A4* and its flanking regions by typing 20 single nucleotide polymorphisms (SNPs) and 7 simple sequence repeat (SSR) polymorphisms in 117 autism trios. When individual markers were analyzed by TDT, 7 SNP markers and 5 SSR markers from either *SLC6A4* or the bleomycin hydrolase gene (*BLMH*) showed nominally significant evidence of transmission disequilibrium. Four markers showed stronger evidence of transmission disequilibrium (TDTmax  $p=0.0005$ ) than 5-HTTLPR. Strong linkage disequilibrium (LD) among markers was found across the region. Thus, support is provided for the hypothesis that either *SLC6A4* itself is a susceptibility gene in autism, or is in linkage disequilibrium with another susceptibility locus (e.g. *BLMH*). Moreover, the specific variant(s) contributing to susceptibility in this region have yet to be elucidated and the strong LD over a large physical region may limit the resolution of fine mapping of loci for autism or other disorders within this gene and surrounding region.

**Dense mapping within the MHC suggests a complex pattern of haplotype associations with rheumatoid arthritis.**

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An initial analysis of 51 microsatellite markers across the HLA region in 257 multiplex families with rheumatoid arthritis (RA) was carried out to identify genetic factors preferentially transmitted with RA, using the single marker pedigree disequilibrium test (PDT). HLA-DRB1\*04 and flanking markers yielded highly significant transmission disequilibrium ( $p < 0.0005$ ) with RA. Several markers telomeric to HLA-DRB1 also displayed highly significant transmission disequilibrium with RA. The most striking of these associations was at the class III/class I border, 150 kb centromeric of HLA-B ( $p < 0.001$ ). A case control analysis of 257 RA probands and 383 controls confirmed these associations. Stratification using HLA-DRB1\*04 and/or markers in close linkage disequilibrium with DRB1\*04 suggests that a subset of these associations are due to effects independent of DRB1\*04. Some of these observations may reflect linkage disequilibrium with DRB1\*01, and others appear to reflect genes present on the common ancestral 8.1 haplotype (A1-B8-DR3). We have further developed an association mining program, based on the Apriori algorithm, to search for HLA marker combinations specifically enriched among RA patients. Overall, the data suggest a complex pattern of associations with particular HLA haplotypes, many but not all of which contain HLA-DRB1 alleles previously associated with RA. In addition, the data suggest the presence of susceptibility genes within the HLA region, outside of the HLA-DRB1 locus. Additional detailed case-control haplotype analysis will be required to fully elucidate these genetic associations.

**Family based association study (qTDT) on lipid abnormality-candidate genes on an isolated, admixed population.** Z. Han<sup>1</sup>, W. Li<sup>1</sup>, D. Shmulewitz<sup>1</sup>, S. Heath<sup>2</sup>, S. Auerbach<sup>3</sup>, M. Blundell<sup>1</sup>, T. Lehner<sup>1</sup>, J. Ott<sup>1</sup>, M. Stoffel<sup>1</sup>, J.M. Friedman<sup>1</sup>, J.L. Breslow<sup>1</sup>. 1) Starr Ctr Human Genetics, Rockefeller Univ, New York, NY; 2) Lab Mathematical Genetics, MSKCC, New York, NY; 3) HRSA, Department of Health & Human Services, New York, NY.

Traditional linkage analyses lack power and precision when applied to complex traits such as cholesterol (chol) level. Association may be more successful at identifying genes of small effect, but are often controversial due to problems such as population stratification (PS). Using quantitative TDT analysis, we examined 8 polymorphic sites, previously shown to be associated with abnormal lipid and lipoprotein levels, on the Kosrae Island where the population is an admixture of native Kosraeans and Caucasian from late 19th century. The 1102 individuals collected in this study were split into 223 nuclear families with total 1527 sibships. The traits studied included triglyceride (Tg), chol, apolipoprotein-AI (apoAI), apolipoprotein-AII (apoAII), apolipoprotein-B (apoB), body mass index (BMI) and blood pressure (BP) levels. The 8 markers were common polymorphisms in following candidate genes: apo AI-CIII-AIV gene cluster (apo CIII/SstI), apo AII (MspI), apo E (HhaI), cholesteryl ester transfer protein (CETP/TaqIB), cholesterol 7 $\alpha$ -hydroxylase (CYP7/BsaI), hepatic lipase (HL/DraI) and microsomal triglyceride transfer protein (MTP/HhpI). The method we used was QTDT program by Abecasis GR et al. When analyzed under a variance component model, the apoCIII/SstI was associated with Tg ( $p=0.0031$ ), and apoE/HhaI was associated with apoB level ( $p=0.0004$ , 0.0070 and 0.0285 for allele E4, E3 and E2). There was suggestive evidence for linkage of apoAII/MspI to systolic BP ( $p=0.0497$ ), and the linkage evidence was accounted for by adding association to the model. Our study has the advantages of reducing the likelihood of false-positive result from PS and increasing the chance of discovering relevant genetic factors due to a more homogenous environmental effect.



**A thorough linkage disequilibrium (LD) analysis in the human angiotensinogen gene (*AGT*).** *T. Nakajima*<sup>1</sup>, *T. Ishigami*<sup>2</sup>, *S. Umemura*<sup>3</sup>, *M. Emi*<sup>4</sup>, *J-M. Lalouel*<sup>2</sup>, *I. Inoue*<sup>1</sup>. 1) Division of Genetic Diagnosis, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; 2) Department of Human Genetics, University of Utah Health Sciences Center, Salt Lake City, UT; 3) Internal Medicine, Yokohama City University, Yokohama, Japan; 4) Department of Molecular Biology, Institute of Gerontology, Nippon Medical School, Kawasaki, Japan.

LD mapping for common diseases explicitly depend on the persistence of LD. To understand the organization of LD in the human gene promises to have great impact on the strategies for identifying candidate genes. *AGT* constitutes the locus that consistently has been associated with essential hypertension (EHT). Variants associated with EHT, T235 and A(-6), vary in frequency among major ethnic groups. Sequencing of angiotensinogen gene in nonhuman primates shows that the T235 and A(-6) variants are fixed in them. The protective M235 and G(-6) variants for EHT, that were in tight LD, are likely to have arisen during the course of human evolution. Haplotypes based on 21 SNPs in *AGT* showed that Caucasian and Japanese shared top four frequent haplotypes, even though the frequencies of them were quite different. In Caucasians G(-6)/M235 haplotype, protective haplotype for EHT, was very common ( $p=0.54$ ). Natural selection might result in the increased frequency of G(-6)/M235 haplotype in Caucasian. We also compared LD parameters  $D'$ ,  $r^2$ , and  $d^2$  based on the data from 861 possible marker pairs in *AGT*. An LD measure  $d^2$  is an appropriate choice for association studies. When compared between  $D'$  and  $d^2$ , the value of  $d^2$  varied widely in the marker pairs with  $D'=1$  or  $-1$ . In cases with  $D'=1$  or  $-1$ , the mean value of  $d^2$  is 0.21 and the proportion of marker pairs with  $d^2<0.1$  (insufficient LD for association studies) is 0.44. These results showed that about half of cases with complete LD in the value  $D'$  display insufficient LD ( $d^2<0.1$ ) for association studies. Another common measure  $r^2$  is compared with  $d^2$ .  $r^2$  and  $d^2$  were significantly correlated. In conclusion, an LD measure,  $r^2$ , is the better parameter than  $D'$  in considering strategies for identifying genes for common disease.

**Linkage disequilibrium mapping and haplotype analysis in the 15q11-q13 autism candidate region reveals suggestive association at *ATP10C* and *OCA2* in the CLSA families.** *E.L. Nurmi, T. Amin, Y. Bradford, M.M. Jacobs, J.L. Haines, J.S. Sutcliffe and the Collaborative Linkage Study of Autism.* Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Autism is a neurodevelopmental disorder with a complex genetic etiology. Observations of maternal duplications affecting chromosome 15q11-q13 in patients with autism and evidence for linkage and linkage disequilibrium to regional markers in chromosomally normal autism families suggest the existence of a susceptibility locus. We have screened the Collaborative Linkage Study of Autism families for a number of single nucleotide polymorphisms within a candidate region covering ~2 Mb and including the Angelman syndrome gene (*UBE3A*), another maternally-expressed gene *ATP10C*, a cluster of GABA<sub>A</sub> receptor subunit genes (*GABRB3*, *GABRA5* and *GABRG3*) and *OCA2*. Transmission disequilibrium test and haplotype analysis methods were applied to the study of these variants. We found suggestive evidence for association of individual polymorphisms and haplotypes at *ATP10C* and *OCA2*. We also detected highly significant increases in allele frequency at the *OCA2* locus in our autism families compared to a control group. *ATP10C*, encoding an aminophospholipid-transporting ATPase, has recently been shown to be imprinted with maternal-specific expression in human brain, thus making it an excellent candidate given the maternal-specificity of chromosomal duplications in association with autistic phenotypes. The *OCA2*, or *P*, gene encodes a putative tyrosine transporter, defects of which affect melanosomal production of melanin and result in hypopigmentation. *OCA2* is also expressed in the brain, where its role is less clear. Ongoing studies will seek to analyze additional polymorphisms and expand haplotype studies in a larger number of families.

**Is the BDNF gene associated with Bipolar Disorder?** *M. Neves-Pereira<sup>1</sup>, E. Mundo<sup>1,2</sup>, P. Muglia<sup>1</sup>, J.L. Kennedy<sup>1,2</sup>, F. Macciardi<sup>1,2</sup>.* 1) Neurogenetics Section, Clarke Division, CAMH, Toronto, ON., Canada; 2) Department of Psychiatry, University of Toronto, Toronto, ON, Canada.

Bipolar Disorder (BP) is a severe psychiatric disease that affects 1% of the population worldwide and it is characterized by recurrent episodes of mania and depression. BP has a strong genetic component with a complex mode of inheritance and heterogeneous clinical presentation. Brain Derived Neurotrophic Factor (BDNF) has been implicated in the pathogenesis of mood disorders. BDNF is reduced in depression and increases with antidepressant treatment. The aim of this study was to test for the presence of linkage disequilibrium between a dinucleotide (CA) polymorphism in the BDNF gene and BP. The CA repeat has 3 common alleles, and is located 1040bp upstream of the starting codon. Two hundred and eighty three triads in which bipolar probands were diagnosed with a semi-structured interview for DSM-IV Axis I diagnoses (SCID) were genotyped. Data was analysed with the Transmission Disequilibrium Test (TDT). Allele 3 was preferentially transmitted to the affected offspring (chi-square=5.765, p=0.02) suggesting that BDNF may confer susceptibility to BP. Future studies involving alternative phenotypes related to BP and other polymorphisms will be conducted to further assess the involvement of BDNF in BP.

**N-Methyl-D-Aspartate Receptor (GRIN1, GRIN2B) in schizophrenia: TDT and case-controls analyses. L.**

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The N-Methyl-D-Aspartate Glutamate receptors (NMDAR) act in the CNS as regulators of the release of neurotransmitters such as dopamine, noradrenaline, acetylcholine and GABA. It has been suggested that a weakened glutamatergic tone increases the risk of sensory overload and of exaggerated responses in the monoaminergic system, which is consistent with the symptomatology of schizophrenia. We have focused on the study of two polymorphisms in GRIN1 and on one polymorphism in GRIN2B. NMDAR1/1 is a G/C substitution localized on the 5' untranslated region; NMDAR1/10 is an A/G substitution localized in exon 6 of GRIN1. There is a T/G substitution in the 3'UTR of GRIN2B. Minor allele frequencies in our sample were calculated to be 0.05, 0.2 and 0.4 respectively. We genotyped 41 nuclear families and 92 ethnically matched case-control pairs. Both samples were collected from the Toronto area. We tested the hypothesis that GRIN1 and GRIN2B polymorphisms were associated with schizophrenia using the Transmission Disequilibrium Test and comparing allele frequencies between cases and controls. The results are as follows: NMDAR1/1,  $c^2 = 0.333$ ,  $p = 0.57$ ; NMDAR1/10,  $c^2 = 0.429$ ,  $p = 0.51$ ; GRIN2B,  $c^2 = 1.19$ ,  $p = 0.2753$ . For the case-control sample: NMDAR1/1:  $c^2 = 0.013$ ,  $p = 0.908$ ; NMDAR1/10:  $c^2 = 0.544$ ,  $p = 0.461$ ; and GRIN2B:  $c^2 = 2.795$ ,  $p = 0.095$ . Although a slight trend was observed for the GRIN2B marker in the case-controls, no significant results were obtained. We are currently collecting additional samples to increase the power of the analyses.

**Haplotypic analysis of COMT polymorphisms and schizophrenia in two Chinese samples.** *V.C. Kodavali<sup>1</sup>, K. Xu<sup>3</sup>, M. Haraczak<sup>1</sup>, M. Cui<sup>3</sup>, T. Li<sup>3</sup>, B.Y. Xie<sup>3</sup>, W. Joel<sup>1</sup>, W.F. Tsoi<sup>4</sup>, N. Saha<sup>4</sup>, V.L. Nimgaonkar<sup>1,2</sup>.* 1) Departments of Psychiatry, University of Pittsburgh, Western Psychiatric Institute and Clinic, 3811 OHara Street, Pittsburgh, PA 15213, USA; 2) Departments of Human Genetics, University of Pittsburgh, Western Psychiatric Institute and Clinic, 3811 OHara Street, Pittsburgh, PA 15213, USA; 3) Guangzhou Psychiatric Hospital, Guangzhou, China; 4) Department of Psychiatry and Pediatrics, National University of Singapore, Singapore.

Catechol-O-methyl transferase (COMT) metabolizes catecholamines such as dopamine, adrenaline and nor-adrenaline. It exists in common high and low activity forms. The low activity form is the result of an amino acid substitution (val-108-met) which reduces the thermostability of the enzyme. Li et al (1996, 2000) reported that the high activity COMT allele, as well as related haplotypes are transmitted more often to patients with schizophrenia among Han Chinese case-control and case-parent samples. To replicate these findings, we have studied three COMT polymorphisms in Chinese cases from Singapore (n = 85), Guangzhou (n = 93) and adult controls (n = 85 and 91 respectively). The polymorphisms investigated were (186 C/T, exon 3), (408 C/G, exon 4) and 472 G/A (Val158Met). We did not find any significant allele or genotype-wise differences in either sample. We also used SNPDM software (Fallin and Schork, 2001) based on the EM algorithm to estimate and compare haplotype frequencies. Significant case-control differences were detected for one of four common haplotypes (186C, 408C, 472G) in the Singapore sample (overall differences in haplotype frequencies chi square = 15.8, p = 0.043). The implications of these findings will be discussed.

**Family-based association studies of schizophrenia in two independent samples with candidate genes DRD3 and NOTCH4.** *V.L. Nimgaonkar<sup>1,2,3</sup>, V.C. Kodavali<sup>1</sup>, S. Prasad<sup>4</sup>, A. Butler<sup>1</sup>, D.E. Downard<sup>1</sup>, F. Zhang<sup>1</sup>, S.N. Deshpande<sup>5</sup>, T. Bhatia<sup>5</sup>, J. Wood<sup>1</sup>, B.K. Thelma<sup>3,4</sup>.* 1) Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh; 2) Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh; 3) Indo-US Collaborative Study of Schizophrenia Genetics, New Delhi; 4) Department of Genetics, Delhi University, South Campus, New Delhi; 5) Department of Psychiatry, Dr Ram Manohar Lohia Hospital, New Delhi.

This study focuses on two putative genetic associations with schizophrenia. An association between the Ser9Gly polymorphism of the dopamine D3 receptor gene (DRD3) and schizophrenia have been reported by several research groups. It is not known whether the Ser9Gly polymorphism alone or a variation in linkage disequilibrium affects susceptibility to schizophrenia. Recently, Wei and Hemmings (2001), reported association of two polymorphisms at Notch4 with schizophrenia. To investigate these findings, we have genotyped four polymorphisms at DRD3 (DRD3,4: a di-nucleotide repeat, -712G/C, -205A/G and S9G) and five polymorphisms in Notch4 (AAGG, TAA, SNP1, SNP2 and CTG) in two independent case-parent samples from India (179) and US (235). The US sample also included a group of unscreened population based controls (n = 93). None of these polymorphisms showed any significant excess transmission of either allele in either population using TDT analysis. We analyzed transmission of haplotypes to cases. The haplotype 712G, -205A and Ser9 at DRD3 showed excess transmission in the US sample but not in the Indian sample ( $p = 0.006$ ). Our results suggest an association at DRD3, which varies by ethnicity.

**Use of DNA pools to assess allele frequencies of single nucleotide polymorphism (SNPs) at a type 2 diabetes mellitus (T2DM) susceptibility locus.** *M.A. Permutt, J.C. Wasson, S. Donelan, G. Skolnick, J. Lin, B.K. Suarez.*  
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Nominal evidence for linkage (Z score of 2.05,  $P < 0.05$ ) was observed on chromosome 20q12-13.1 in Ashkenazi Jewish multiplex families with T2DM. Combined analysis by the International T2DM Linkage Consortium has identified a peak at D20S107. To identify the gene(s) contributing to susceptibility in this region, linkage disequilibrium mapping is being employed. We determined that the most efficient, cost-effective method of genotyping SNPs was by the use of DNA pools with the PSQ96 Pyrosequencer and Allele Quantitation software. Accuracy of allele frequency estimation was assessed by mixing two different homozygous DNAs in various proportions at 5% increments. The observed vs. actual allele frequencies had a correlation coefficient of 0.9963. Pools of DNA from each of 150 diabetics and 150 non-diabetics were precisely assembled. With these pool sizes, differences between the groups of  $>7\%$  would be significant at the  $p < 0.05$  level, and could then be replicated with additional pools. To assess the actual genotype frequencies compared to those observed in the pools, each individual DNA was genotyped and the combined frequencies compared with those of the DNA pools. Four SNPs were tested for both cases and controls and the differences ranged from 0 to 1.7%. We are examining SNPs in one lod intervals around D20S107 containing 7.4Mb of DNA with 30 known or predicted genes ([www.ncbi.com](http://www.ncbi.com)). SNPs were selected from the SNP Consortium Database ([snp.cshl.org/](http://snp.cshl.org/)), although none of these SNPs had been previously validated. Examination of 11 of 15 of these SNPs revealed minor allele frequencies  $>20\%$ . Comparing allele frequencies between cases and controls, the greatest difference noted has been 7% ( $p=0.055$ ). We conclude that with this method we can rapidly and accurately determine differences in allele frequencies between cases and controls for a large number of SNPs across this chromosomal region.

**Family based and case-control associations serotonergic polymorphisms in Bipolar I Disorder.** *S. Ranade<sup>1</sup>, L.K. Brar<sup>1</sup>, V.C. Kodavali<sup>1</sup>, J. Wood<sup>1</sup>, H. Mansour<sup>1</sup>, B. Devlin<sup>1,2</sup>, D.J. Kupfer<sup>1</sup>, V.L. Nimgaonkar<sup>1,2</sup>.* 1) Department of Psychiatry, University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania, USA; 2) Department of Human Genetics, University of Pittsburgh School of Medicine, Western Psychiatric Institute and Clinic, Pittsburgh, Pennsylvania, USA.

**Introduction:** The genetic factors contributing susceptibility to Bipolar Disorder I (BD I) are unknown. The mode of inheritance of BD I is complex and individual genetic susceptibility factors may contribute only a fraction of the liability. Therefore, we are presently conducting association studies using candidate gene polymorphisms. Since association studies are critically dependent on the choice of controls, we have employed unrelated as well as family based controls. **Methods:** We report on 80 nuclear families having a proband with BD I (DSM IV criteria), as well as available parents. Cord blood samples from local live births served as unrelated, unselected controls (n = 90). To enable haplotype-based analyses, five polymorphisms were analyzed at the serotonin 2A receptor (5-HT<sub>2A</sub>) and two polymorphisms for serotonin transporter (SLC6A4) loci. The HT<sub>2A</sub> markers included single nucleotide polymorphisms (Pro2, Thr25Asn, T102C, C516T and His452Tyr) while the SLC6A4 markers included an insertion/deletion polymorphism in the promoter sequence and variable number or tandem repeat polymorphism (VNTR) in the second intron. Associations with both genes have been suggested previously. **Results:** Significant transmission distortion was detected with the His452Tyr polymorphism (Transmission Disequilibrium Test,  $p < 0.005$ , 1 df). Haplotypes using the TC102, CT516 and Thr25Asn polymorphisms also displayed transmission distortion, but no case-control differences in genotype counts or allele frequencies were noted when the unrelated controls were analyzed. Our results support a plausible association of BD I with 5-HT<sub>2A</sub>. Analyses employing larger samples are warranted.



**Two-locus model of inheritance of multiple sclerosis in a large North American pedigree.** *E. Vitale<sup>1,6</sup>, S. Cook<sup>2</sup>, R. Sun<sup>1</sup>, C. Specchia<sup>3</sup>, K. Subramanian<sup>1</sup>, M. Rocchi<sup>4</sup>, D. Nathanson<sup>5</sup>, M. Schwalb<sup>1</sup>, M. Devoto<sup>3</sup>, C. Rohowsky-Kochan<sup>2</sup>.* 1) Dept Microbiology & Molecular Genetics, UMDNJ New Jersey Medical School, Newark, NJ; 2) Dept of Neuroscience, UMDNJ-New Jersey Medical School, Newark, NJ; 3) Dept of Research, AI duPont Hospital for Children, Wilmington DE; 4) Dept of Cytogenetics University of Bari, Italy; 5) Dept of Neuroscience, Sacred Heart Hospital Allentown, NJ; 6) CNR Institute of Cybernetics, Naples, Italy.

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) with a probable immune-mediated pathogenesis. Strong evidence supports the hypothesis that MS is determined by genetic and environmental factors, but these factors remain largely undefined. Several studies have shown association of MS with the HLA class II region, specifically DR15, DQ6. However, there is no convincing evidence of a common susceptibility locus. We have identified a pedigree of Pennsylvania Dutch extraction, in which MS segregates with an autosomal dominant inheritance pattern. We have collected blood samples from 18 family members, seven of whom show typical signs of MS lesions by MRI. The 18 individuals were serotyped for HLA class I and II and analyzed by a genome wide screen for linkage analysis. We have found evidence for suggestive linkage to markers on 12p12 with a maximum multipoint lod-score of 2.71 conditional on the presence of DR15, DQ6. The significance of the association of MS with both the HLA-DR15 allele and the chromosome 12p12 haplotype was further tested by contingency table analysis. This results in a chi-square of 16 and a p-value of 0.00011 from Monte Carlo simulation analysis. A potentially interesting gene in the critical region is GD3 synthase which is part of the sialyltransferase family characterized by having the sialyl motif and a key regulatory domain that controls the ganglioside biosynthesis pathway. In search for a mutation we sequenced the 4 exons and part of the intronic sequences characterizing GD3 gene, and we found a SNP at position +30 of a splice donor site of intron 3 in our patients. We are in the process of assaying this change.

**Genetic Analysis of the CTLA-4/CD28 Gene Region in Type I Diabetes.** *M. Barreto<sup>1</sup>, A. Vicente<sup>1</sup>, T. Vassilevskaya<sup>1,2</sup>, N. Duarte<sup>1</sup>, L. Gardete-Correia<sup>2</sup>, J. Boavida<sup>2</sup>, R. Duarte<sup>2</sup>, F. Raposo<sup>2</sup>, J. Mendes<sup>2</sup>, C. Penha-Gonçalves<sup>1</sup>, D. Holmberg<sup>1</sup>.* 1) Instituto Gulbenkian Ciência, Oeiras, Portugal; 2) Associação Protectora dos Diabéticos de Portugal.

Insulin Dependent Diabetes Mellitus (IDDM) is a polygenic disease, characterized by the autoimmune destruction of the insulin-producing  $\beta$  cells within the pancreatic islets. The objective of this study is the identification and characterization of genes involved in the etiology of IDDM, focusing on the IDDM12 susceptibility region on 2q33-34. Although linkage and/or association of IDDM to this locus has been reported, the gene(s) involved have not yet been identified. Two strong candidate genes map to this area, CD28 and CTLA-4, which are part of the co-stimulatory system involved in the activation of T cells. It is widely accepted that dysregulation of co-stimulation contributes to the initiation and maintenance of autoimmunity due to activation of self-reactive T cells. To further define the IDDM12 susceptibility region, we performed a genetic screening using 11 polymorphic markers in the 2q33-34 chromosomal region, in a Portuguese population sample of 120 patients and 150 matched controls. We have confirmed a significant association of the disease with markers within the CTLA-4 gene (a SNP involving an A to G transition in the promoter ( $p = 0.021$ ) and a microsatellite marker in the 3' UTR ( $p < 0.001$ )). We also noticed that the longer alleles at the dinucleotide repeat located in the 3' UTR are associated with increasing risk for IDDM. None of the other 7 markers tested, spanning the region around and between CTLA-4 and CD28, showed any association.

**Evidence for association of the BCL3 gene in 98 Brazilian nonsyndromic clefting patients using the transmission disequilibrium test (TDT).** D.A. Gaspar<sup>1,2</sup>, R.C. Pavanello<sup>1,2</sup>, B.C. Araujo<sup>3</sup>, M. Andre<sup>4</sup>, S. Steman<sup>5</sup>, S.R. Matioli<sup>2</sup>, M.R. Passos-Bueno<sup>1,2</sup>. 1) Centro de Estudos do Genoma Humano; 2) Depto de Biologia, IBUSP; 3) HMJ; 4) FOU SP; 5) FMUSP, Sao Paulo, Brasil.

Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is a common malformation with an incidence of at least 1 in 1000 caucasian live births. The inheritance of CL/P is complex and approximately 20 genes interacting multiplicatively along with environmental factors have been considered as responsible for this phenotype. Stein *et al.* . 1995 (Am J Hum Genet 57:257-272), using linkage analysis in multiplex families, first presented evidence that the BCL3 gene or a nearby locus at 19q13.2 might be involved with NSCL/P. Some other studies based on association analysis between 19q13.2 markers and NSCL/P also supported this idea. In this study, we analysed two markers from 19q13.2 region (D19S178 and an intragenic marker in BCL3) in 98 families with at least one NSCL/P patient (45 familial cases and 53 isolated families). Smoking habits were assessed in 72 mothers of NSCL/P patients (23 smokers; 49 nonsmokers). The TDT test showed a significant excess transmission of the 131bp allele (54 alleles transmitted vs. 35 nontransmitted; P=0.04) at the BCL3 locus while the 167 bp allele of D19S178 (5 alleles transmitted vs. 15 nontransmitted; P=0.025) was under-represented in NSCL/P patients. A specially developed TDT test based on random permutations considering the two loci simultaneously was applied to these data and we observed that one haplotype (BCL3/D19S178: 131bp/169bp) was more frequently transmitted to NSCL/P patients (15 haplotypes transmitted vs. 6 nontransmitted; P=0.052), suggesting a marginal association between BCL3 gene and NSCL/P in our sample. A preliminary analysis did not suggest an interaction of smoking and the BCL3 locus in the predisposition of clefting in our families. Our analysis, if taken together with other studies, further supports that the BCL3 gene or a nearby locus might play a role in the predisposition of NSCL/P, but probably in a small proportion of cases. FAPESP, HHMI, PRONEX, CNPq.

**The role of human circadian clock genes in bipolar disorder.** *C.M. Nievergelt, D.F. Kripke, J.R. Kelsoe.* Dept Psychiatry, 0603, Univ California, San Diego, La Jolla, CA.

Bipolar disorder is a complex genetic human disease with strong evidence for heritability and has been a major focus of linkage studies in psychiatry. Linkage reports have implicated fairly broad genomic regions, thus making gene identification a challenging project. An alternative to whole-genome surveys is the analysis of specific candidate genes. Bipolar disease is suspected to be associated with malfunctions of the circadian system, which regulates individual circadian rhythms and enables the adaptation to a daily 24-hour cycle and seasonal changes. Several human clock genes have been identified and localized and a comparison with linkage hotspots for bipolar disorder has revealed close correspondences.

One of the most promising candidates, human cryptochrome 1 (hCRY1; located on 12q23 q24.1) was analyzed here. Linkage of bipolar disorder to 12q has been reported by several groups. Using two hCRY1 flanking microsatellite markers, linkage analysis revealed slightly positive LOD scores under a dominant transmission model with medium penetrance in 23 out of 52 genotyped bipolar families. Affected subjects from these families and controls (n=28) were screened for mutations by sequencing 13 kb (13%) of the genomic sequence. We identified a total of 16 single nucleotide polymorphisms (SNPs) and a 3bp insertion in the promoter region (2 SNPs), the 5' and 3' untranslated regions (4 and 2 SNPs, respectively), and the 12 exons and their flanking regions (1 and 8, respectively). However, no mutations with functional impact in exonic regions were found. SNP frequencies ranged between 0.02 and 0.37. These SNPs will be useful for linkage disequilibrium studies of this gene in bipolar disorder. Such studies are now underway in a set of 150 parent proband triads.

We are also now employing a similar strategy for several other human clock genes that map to regions reportedly linked to bipolar disorder.

**Possible relationship between the van der Woude syndrome (vWS) locus and nonsyndromic cleft lip with or without cleft palate.** *C. Houdayer<sup>1</sup>, C. Bonaiti Pellié<sup>2</sup>, C. Erguy<sup>1</sup>, V. Soupre<sup>3</sup>, L. Bürglen<sup>4</sup>, R. Couderc<sup>1</sup>, MP.*

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Cleft lip with or without cleft palate (CL/P) is one of the commonest congenital malformation conditions in humans which may be part of a defined syndrome, sequence or association, although most individual or familial cases present as an isolated (nonsyndromic) malformation defect (NSCL/P). Inheritance is generally regarded as multigenic although, in some families, NSCL/P seemingly segregates as a monogenic trait. On the other hand, van der Woude syndrome (vWS) is a rare autosomal dominant with cardinal features of lower-lip pits (LLP) and CL/P or cleft palate (alone). Since none of these traits is present in all mutation carriers, some individual or familial vWS cases, especially those lacking LLP, are indiscernible from NSCL/P, raising the question whether allelic variation at the vWS locus could underlie NSCL/P. This question was addressed using parametric linkage (LOD score) analysis in 21 multiplex NSCL/P families based on a tightly linked microsatellite marker (D1S3753), and nonparametric analysis using the transmission/disequilibrium test (GTDT) in 106 NSCL/P triads and selecting markers D1S205, D1S491, and D1S3753. No evidence for linkage of NSCL/P to vWS was found on the 21 families using the LOD score approach. In contrast, TDT yielded a significant P value of 0.04 for D1S205, supporting involvement of vWS in NSCL/P in a complex, modifying/polygenic manner rather than as a monogenic/major disease locus.

**Cloning and Expression Analysis of a Novel Candidate Gene for Bipolar Disorder and Schizophrenia on Human**

**Chromosome 18p11.2.** *L.D. McNabb<sup>1</sup>, J.C. Friedland<sup>1</sup>, T.A. Gieringer<sup>2</sup>, K. Rojas<sup>3</sup>, J. Overhauser<sup>3</sup>, G. Smith<sup>4</sup>, G. Golden<sup>4</sup>, S. Arnold<sup>2</sup>, T.N. Ferraro<sup>1,2</sup>, W.H. Berrettini<sup>1,2</sup>, R.J. Buono<sup>2</sup>.* 1) Department of Pharmacology, University of Pennsylvania, Philadelphia, PA; 2) Department of Psychiatry, University of Pennsylvania, Philadelphia, PA; 3) Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, PA; 4) VAMC, Coatesville, PA.

A locus on human chromosome 18p11.2 has been identified through linkage and association studies as containing a possible candidate gene for bipolar disorder and/or schizophrenia. cDNA selection and direct sequencing have identified several novel DNA sequences that are likely to be expressed. One sequence in particular, 1B10, is in close proximity to a microsatellite marker (D18S53) that has been linked with schizo-affective illness. A 3.9 kilobase cDNA clone was identified using PCR analysis with primers specific to 1B10. This clone, named 22444, was expressed only in brain tissue, and contained the entire 1B10 sequence, as well as D18S53 in the 3 prime untranslated region. Rabbit polyclonal antibodies generated against the predicted amino acid sequence of clone 22444 were used in western blots to identify a protein from human tissues. The predicted amino acid sequence of 22444 leads to the formation of a 14 kilodalton protein; however, polyclonal serum #1363 recognizes a specific 20 kilodalton protein in human brain tissue. This protein is not detected by western blot analysis in peripheral tissues, which is in agreement with the PCR findings. We are currently using this polyclonal antibody to isolate the 30 kilodalton protein for sequencing in order to provide evidence that 22444 is translated into protein. In addition, mutation scanning of the DNA sequence is ongoing to identify variants found in ill versus well individuals. This research was supported by a NARSAD Young Investigator Award to RJ Buono.

**Analysis of a missense mutation in the catatonic schizophrenia candidate gene WKL1 in bipolar affective disorder and schizophrenia patients from Denmark and the Faroe Islands.** *M.D. Lundorf<sup>1</sup>, T.H. Jorgensen<sup>2</sup>, H.N. Buttenschoen<sup>2</sup>, A.G. Wang<sup>3,4</sup>, M. Vang<sup>3</sup>, T.A. Kruse<sup>5</sup>, O. Mors<sup>2</sup>, H. Ewald<sup>1,2</sup>.* 1) Department of Biological Psychiatry, Institute for Basic Psychiatric Research, Risskov, Aarhus, Denmark; 2) Department of Psychiatric Demography, Institute for Basic Psychiatric Research, Psychiatric Hospital in Aarhus, DK-8240 Risskov, Denmark; 3) Department of Psychiatry, National Hospital, Torshavn, Faroe Islands; 4) Copenhagen University Hospital, Denmark; 5) Department of Clinical Biochemistry and Genetics, Odense University Hospital, Denmark.

Genetic mapping studies, investigations of cytogenetic abnormalities and direct examination of candidate genes suggest that chromosome 22q may harbour risk genes for schizophrenia as well as bipolar affective disorder. Patients from a genetic isolate of the Faroe Islands were typed for 35 evenly distributed polymorphic markers on 22q in a search for shared risk genes in the two disorders. A region on 22q13.3 showed increased sharing of haplotypes in bipolar and schizophrenic patients. Of particular interest is a segment close to the WKL1 gene recently suggested to harbour a missense mutation in individuals with catatonic schizophrenia. We evaluated this mutation by a primer extension assay in 13 bipolar affective disorder patients, 11 schizophrenic patients and 40 controls from the Faroe Islands. Furthermore, the polymorphism was tested in 79 bipolar affective disorder patients and 84 controls from Denmark. However, we did not detect the mutation in any of the 454 chromosomes tested. This suggests that the WKL1 missense mutation is very rare in, or absent from, the Danish and Faroese populations and it is not associated with schizophrenia or bipolar affective disorder in our samples.

**Testing Autism Candidate Genes on Chromosome 7.** *H.B. Hutcheson<sup>1</sup>, Y. Bradford<sup>1</sup>, S.E. Folstein<sup>2</sup>, M.B. Gardiner<sup>1</sup>, J. Piven<sup>3</sup>, S.L. Santangelo<sup>2,4</sup>, J.S. Sutcliffe<sup>1</sup>, V. Vieland<sup>5</sup>, T.H. Wassink<sup>6</sup>, J.L. Haines<sup>1</sup> and Collaborative Linkage Study of Autism (CLSA).* 1) Program in Human Genetics, Vanderbilt University Medical Center, Nashville, TN; 2) Department of Psychiatry, New England Medical Center/Tufts University School of Medicine, Boston, MA; 3) Neurosciences Hospital, University of North Carolina School of Medicine, Chapel Hill, NC; 4) Harvard University School of Public Health, Boston, MA; 5) Division of Statistical Genetics, University of Iowa Colleges of Public Health and Medicine, Iowa City, IA; 6) Psychiatry Research, University of Iowa College of Medicine, Iowa City, IA.

Based on previous genetic and cytogenetic studies performed in autism, evidence points to the existence of one or more autism genes residing on chromosome 7q (115cM-149cM on the Marshfield map). However, further localization using linkage analysis has proven difficult. To overcome this problem, we examined our Collaborative Linkage Study of Autism (CLSA) dataset to identify only the families potentially linked to chromosome 7. 47 from a total of 86 families were identified and 17 markers were used to generate chromosomal haplotypes. We performed recombination breakpoint analysis to determine if any portion of the chromosome was predominately shared. The preponderance of these data identified a 6 cM region between D7S501 and D7S2847 as being most commonly shared. Additional markers at 1 cM intervals within this region were genotyped and association and recombination breakpoint analysis was performed. Although no significant association was found, the preponderance of the recombination breakpoint data points to a shared region between D7S496-D7S2418 (120 cM-123 cM) encompassing about 4.5 Mb of genomic DNA with over 50 genes. Multiple neuronally expressed genes, such as KIAA0716 and Leucine Rich Repeat Protein, have been prioritized for analysis. A minimum of five single nucleotide polymorphisms (SNPs) was examined in these genes but none of the analyses resulted in any significant allelic or haplotypic associations. Thus we conclude that these two genes are not involved in autism and examination of additional genes in this region is needed and underway.



**Genetic analysis of tryptophan hydroxylase gene polymorphisms in a chromosome 11p15 candidate region for autism.** *M.M. Jacobs, E.L. Nurmi, Y. Bradford, T. Amin, J.L. Haines, J.S. Sutcliffe and the Collaborative Linkage Study of Autism.* Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Reports of serotonergic abnormalities in both autistic probands and their relatives and an elevated prevalence of depression, anxiety, and obsessive-compulsive disorder in relatives of autistic subjects may reflect an underlying involvement of the molecular mediators of serotonergic neurotransmission in the pathogenesis of autism. Further, drugs that alter serotonin signaling can be used to treat anxiety, social phobia, and obsessive-compulsive disorder in both autistics and in the general population. A genomic linkage screen and follow-up analysis of the Collaborative Linage Study of Autism families identified a candidate region on chromosome 11p with a peak two-point LOD score of 2.04 at marker D11S1999 at 17 cM. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step in the synthesis of serotonin; its gene is located nearby D11S1999 at ~21 cM and is therefore an attractive functional candidate for involvement in autism susceptibility. In order to analyze *TPH* as a candidate gene to account for genetic linkage to this region, we have selected single nucleotide polymorphisms (SNPs) within the transcriptional unit. SNPs were genotyped in a subset of the CLSA families and analyzed using a variant of the transmission disequilibrium test, which is appropriate for use with sibpair datasets. Initial analysis of one SNP within intron 2 and two others flanking the transcriptional unit does not show evidence for allelic association. Other functional candidate genes within a broader region around D11S1999 include the dopamine D4 receptor, tyrosine hydroxylase, and secretin. Ongoing studies seek to analyze additional polymorphisms in *TPH* and other positional candidates individually and within haplotypes to investigate potential association of gene variants with autistic disorder.

**The HLA/TNFA region is linked to leprosy clinical subtypes.** *M.T. Mira*<sup>1</sup>, *A. Alcaïs*<sup>2</sup>, *N.V. Thuc*<sup>3</sup>, *T. Huong*<sup>3</sup>, *M.C. Phuong*<sup>3</sup>, *L. Abel*<sup>2</sup>, *E. Schurr*<sup>1</sup>. 1) Centre for Host Resistance, McGill University, Montreal, Quebec, Canada; 2) INSERM, U 550, Hopital Necker, Paris, France; 3) Hospital for Dermato-Venereology, Ho-Chi-Minh City, Vietnam.

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The disease presents as a spectrum of clinical manifestations ranging from paucibacillary (PB), tuberculoid forms to multibacillary (MB), lepromatous forms. We have enrolled 20 mixed PB and MB multiplex nuclear families from South Vietnam for a genetic study of leprosy susceptibility. These families have been used in a previous linkage study that identified the NRAMP1 region as one important factor controlling primary steps of host responsiveness to infection by *M.leprae*. In the present study, our objective was to evaluate linkage of additional candidate regions to leprosy susceptibility. DNA samples were genotyped for biallelic single-nucleotide polymorphisms (SNPs) and for highly informative microsatellite markers closely linked to the vitamin D receptor encoding gene (VDR, chromosome 12q13), the tumour necrosis factor alpha gene (TNFA) located within the HLA complex (HLA/TNFA, chromosome 6p21), the TNFA-receptor 1 and -receptor 2 genes (TNFR1, chromosome 12p13 and TNFR2, chromosome 1p36), as well as for informative markers within the cytokine gene cluster on chromosomal region 5q33-q35. Genetic model-free sib-pair linkage analysis was performed using the MLB method that allowed simultaneous consideration of PB and MB siblings within the same family. No significant linkage was found between any of the candidate gene regions and leprosy per se. Conversely, we observed significant linkage between leprosy clinical subtypes and the HLA/TNFA region (corrected multipoint p-value =  $8 \times 10^{-4}$ ). The estimated proportion of parental haplotypes shared by two concordant sibs (both PB or both MB) was 0.77. The estimated proportion of haplotypes shared by discordant sibs (one PB and one MB) was 0.23. These results support the general concept that NRAMP1 and other innate resistance genes control initial steps in leprosy pathogenesis, while HLA/TNFA linked immune response genes determine the specific clinical manifestations of the disease.

**Lack of Association Between HoxA1, HoxB1, Reelin and WNT-2 Gene Variants and Autism in 110 Multiplex Families.** *J. Li<sup>1</sup>, H.K. Tabor<sup>1</sup>, L. Nguyen<sup>1</sup>, C. Gleason<sup>1</sup>, L.J. Lotspeich<sup>2</sup>, D. Spiker<sup>2</sup>, N. Risch<sup>1</sup>, R.M. Myers<sup>1</sup>.* 1) Dept Genetics, Stanford Univ. School of Medicine; 2) Psychiatry and Behavioral Sciences, Stanford Univ, Stanford, CA 94305.

Autism is a severe neurodevelopmental disorder characterized by marked social deficits, delay and deviance in language development and communication skills, and a restricted range of stereotypical, repetitive behaviors and limited interests. The etiology of autism is largely unknown. However, family and twin studies have provided compelling evidence for a strong genetic component in most idiopathic cases. Several recent candidate gene studies separately suggested that the HoxA1, HoxB1, Reelin and WNT-2 genes play a role in susceptibility to autism. To determine whether these findings could be replicated, we screened these genes for DNA polymorphisms by sequencing all exons and adjacent intronic regions in 24 individuals with autism. We identified one single base substitution variant in HoxA1, a common haplotype in HoxB1 comprising three single nucleotide polymorphisms (SNP), a polymorphic trinucleotide repeat in the 5' Untranslated Region (UTR) of Reelin, and four common SNPs in WNT-2, of which one is in the 5' UTR, and three are in the 3' UTR. We genotyped all these polymorphisms in 110 multiplex families with autism, and applied the Transmission Disequilibrium Test to detect possible association of these variants to autism. Our results demonstrated no deviation from the null hypothesis of no association. We have also separately examined transmissions (i) within individual mating types, (ii) for paternal versus maternal alleles, (iii) to affected versus unaffected children, and (iv) for transmission to affected boys versus girls. None of these subsets revealed significant deviation from the null expectation. Our interpretation of these findings is that it is unlikely that HoxA1, HoxB1, Reelin and WNT-2 play a significant role in the genetic predisposition to autism.

**The N-Methyl-D-Aspartate receptor subunit 2B gene (GRIN2B) is not linked to Bipolar Disorder: evidence for specificity of the role of GRIN1.** *E. Mundo<sup>1</sup>, N. Prociw<sup>1</sup>, N. King<sup>1</sup>, F. Ajmar<sup>2</sup>, E. De Maria<sup>2</sup>, P. Mandich<sup>2</sup>, F.*

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The glutamate system has been involved in the pathogenesis of schizophrenia (Carlsson et al, 1999) and the most common mood stabilizers used to treat Bipolar Disorder (BP) appear to act via the glutamate system (Dixon and Hokin, 1997; Loscher, 1999). The N-Methyl-D-Aspartate (NMDA) receptor subtype of the glutamate-gated ion channels is composed of different combinations of sub-units (Monyer et al, 1992). In a recent family-based association study, we reported a significant association, confirmed by a haplotype analysis, between the N-Methyl-D-aspartate receptor subunit 1 gene (GRIN1) and BP (Mundo et al, 2001). The aim of the present study was to investigate the same sample for the presence of linkage disequilibrium between the gene encoding for the subunit 2B of the N-Methyl-D-aspartate receptor (GRIN2B) (Mandich et al, 1994) and BP. One hundred and eighteen BP I or BP II probands with their living parents were studied. Diagnoses were assessed by a structured interview for DSM-IV (APA, 1994) (SCID-I). Genotyping data of the BstYI (G/T) polymorphism of the GRIN2B were analyzed using the Transmission Disequilibrium Test (TDT) (Spielman et al, 1993). Sixty-one triads had heterozygous parents and were suitable for the final analyses. The two alleles of the BstYI polymorphism were transmitted to the probands with similar frequency ( $\chi^2=0.610$ ,  $df=1$ ,  $p=0.435$ ). These negative results for the GRIN2B appear to confer specificity to the GRIN1 as a susceptibility marker for BP. Further investigations on larger samples and on alternative phenotypes related to BP (e.g., response to mood stabilizers) are warranted.

**Dopamine system genes, sensation seeking, and the risk for substance use disorders.** *M.M. Vanyukov<sup>1,2,3,5</sup>, B.S. Maher<sup>4,5</sup>, R.E. Ferrell<sup>2,5</sup>, L. Kirisci<sup>1,3,5</sup>, H.F. Simkevitz<sup>1,5</sup>, G.P. Kirillova<sup>1,5</sup>, B. Devlin<sup>3,5</sup>, M.L. Marazita<sup>1,2,4,5</sup>, R.E. Tarter<sup>1,3,5</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.

The reward pathways for different drugs share the structures of the brain comprising the mesocorticolimbic dopaminergic system (Koob & Le Moal, *Neuropsychopharmacology* 24:97-129, 2001). Dopaminergic mechanisms are also involved in natural reward, especially under conditions of novelty. There are data indicating that mesolimbic dopamine neurons code for stimuli with high motivational impact due to their novelty, rather than for generic motivational salience (Bassareo & Di Chiara, *J Neurosci* 17:851-861, 1997). The relationship between parental substance use disorder (SUD) and offspring's risk for SUD is in part mediated by offspring's sensation (novelty) seeking (Kirillova et al., *J Child Adol Substance Abuse*, in press). We applied the transmission/disequilibrium test for quantitative traits (QTDT) to the relationship between dopamine system genes (dopamine transporter and dopamine D2, D4, and D5 receptor genes) and the scales of Zuckerman's Sensation Seeking Questionnaire (SSQ) and the risk for SUD in a sample of Caucasian boys and their parents. Significant associations were detected between a microsatellite polymorphism at the dopamine D2 receptor gene and all four SSQ scales. A QTDT analysis of the single factor derived from the scales, however, showed its significant association with a VNTR at the dopamine transporter (DAT1) gene. Controlling the SSQ scales for the common factor did not affect their associations with the DRD2 gene, indicating independent contributions of the two genes to the common sources of variance in the scales. A QTDT analysis of a quantitative index of the liability to SUD derived for 10-12 year old boys using item response theory (Vanyukov & Tarter, *Drug Alcohol Dep* 59:101-123, 2000) showed a trend toward an association with the DAT1 polymorphism. This trend is consistent with significant differences observed for this polymorphism between adult SUD and control males.

**Polymorphisms of interferon gamma (*IFNG*) contribute to gender-based differential susceptibility to MS.** B.G. Weinshenker<sup>1</sup>, O.H. Kantarci<sup>1</sup>, A. Goris<sup>6</sup>, S. Heggarty<sup>4</sup>, M.G. Marrosu<sup>7</sup>, C. Graham<sup>4</sup>, S. Hawkins<sup>5</sup>, E. Atkinson<sup>2</sup>, M. de Andrade<sup>2</sup>, K. Vandebroek<sup>3</sup>. 1) Dept Neurology, Mayo Clinic, Rochester MN; 2) Dept of Health Sciences Research, Mayo Clinic, Rochester MN; 3) School of Pharmacy, Queen's University of Belfast, Belfast UK; 4) Dept of Medical Genetics, Queen's University of Belfast, Belfast UK; 5) Dept of Neurology, Royal Victoria Hospital, Belfast UK; 6) Rega Institute for Medical Research, University of Leuven, Belgium; 7) Dept of Neuroscience, University of Cagliari, Cagliari, Italy.

Multiple sclerosis (MS) is a complex disease that is twice as common in women than men. *IFNG* is a T helper 1 cytokine, the expression of which increases preceding exacerbations of MS. Expression of *IFNG* is higher in women than in men with diverse inflammatory and infectious diseases, including MS. An intron 1 microsatellite is associated with *IFNG* expression. We tested this and other novel intragenic polymorphisms of *IFNG* uncovered by dideoxyfingerprinting for population and family-based association with susceptibility to MS. We found that the frequency of the 12 CA-repeat allele or of the 12/12 genotype was lower in men with MS compared to controls. The odds ratios for carriers of the 12 repeat allele was 0.26 (95% CI: 0.10-0.69) in Olmsted County, MN and 0.15 (0.03-0.79) in Sardinia (DR3/4-, low risk by MHC). For those with the 12/12 repeat genotype, the odds ratio was 0.16 (0.04-0.68) in Olmsted County, 0.06 (0.01-0.49) in Sardinia (DR3/4-) and 0.38 (0.16-0.92) in Northern Ireland. Similar findings were observed for the G allele of an exon 4 position 325 (untranslated region) G/A single nucleotide polymorphism that is in linkage disequilibrium with the 12 CA-repeat allele of the microsatellite in Olmsted County. The 12 repeat allele was transmitted less frequently than expected from heterozygous parents to affected Sardinian DR3/4- men but not women. A subsequent linkage disequilibrium survey using multiple microsatellites localized the responsible locus to a 94-kb interval immediately surrounding *IFNG*. The lower frequency of the high expression alleles of *IFNG* in men destined to develop MS may explain the relative protection of men from MS.

**Analysis of GABAA receptor subunit genes in Alcohol Dependence and Obsessive-Compulsive Disorder.** *M. Radel, R. Vallejo, R. Aragon, J.C. Long, M. Virkkunen, D. Goldman.* Lab Neurogenetics, NIAAA/NIH, Rockville, MD.

The major objective of this study was to evaluate, using polymorphism detection and linkage strategies, the role of genetic variation of GABAA receptor genes in the genetic susceptibility to alcohol dependence (AD). In addition, the role of GABRG2 in the genetic susceptibility to obsessive-compulsive disorder (OCD), a severe anxiety disorder, was analyzed. Sequence variants of the GABRA1, GABRA6, GABRB2 and GABRG2 genes, on chromosome 5q, and of GABRB1 on 4p, were detected by pDHPLC. The sensitivity and specificity of this novel method was first tested in double-blind fashion across 9 known single nucleotide polymorphisms (SNPs). Putative variants were confirmed and characterized by automated sequencing. A panel of six SNPs at the 5q cluster was selected for linkage and association studies and genotyped blind to diagnosis by PCR-RFLP. For AD, two psychiatrically interviewed samples from population isolates were used: A Southwestern American-Indian (SWAI) tribe (N=433) and a Finnish sample (N=473). There was no deviation of genotypic distributions from Hardy-Weinberg (HW) equilibrium. Association of GABRB2 and GABRA6 alleles with AD was detected in both samples. Sib-pair linkage of GABRG2 to AD was detected in the Finns. Linkage disequilibrium (LD) between alleles at the six loci did not vary as a simple function of distance, and normalized LD coefficients varied between them. The trimmed haplotype analysis showed evidence of linkage of 5q GABAA haplotypes to AD. These results are consistent with genetic variation at or near the 5q GABAA cluster conferring differential susceptibility to alcohol dependence. To investigate the role of the GABRG2 gene in OCD, subjects and controls from the US (N=96) and Italy (N=92) were genotyped blind to diagnoses for two GABRG2 SNPs. There was no H-W deviation. Population association was detected between alleles at both SNPs and OCD in both samples. These results suggest that either these polymorphisms alter function, or that they are in linkage disequilibrium with a functional variant that confers increased genetic vulnerability to OCD.

**Association study of SNPs for the FCHL locus on 1q21.** *P. Pajukanta*<sup>1</sup>, *M.-R. Taskinen*<sup>2</sup>, *J.S. Sinsheimer*<sup>1,3</sup>, *J.S. Bodnar*<sup>1</sup>, *A.J. Lysis*<sup>1</sup>, *L. Peltonen*<sup>1,4</sup>. 1) Dept of Human Genetics, UCLA, USA; 2) Dept of Medicine, University of Helsinki, Finland; 3) Dept of Biomathematics, UCLA, USA; 4) Dept of Molecular Medicine, National Public Health Institute, Finland.

Familial combined hyperlipidemia (FCHL) is a common familial lipid disorder predisposing to coronary heart disease. Despite intensive efforts, the etiology of FCHL has remained unknown. We previously identified a locus for FCHL on human chromosome 1q21-q23 in 31 Finnish FCHL families. The finding has been replicated in FCHL families from other, more heterogeneous populations, and the same 1q21 region has also been linked to non-insulin-dependent diabetes mellitus (NIDDM) in several studies, suggesting a partially shared genetic background for FCHL and NIDDM. Interestingly, a locus for combined hyperlipidemia (Hyplip1) in mouse was mapped to a region on chromosome 3 that was potentially orthologous to human chromosome 1q21. Fine mapping of the Hyplip1 gene assigned the human counterpart 10 Mb of the peak linkage markers to FCHL on 1q21. To determine whether the same gene is responsible for hyperlipidemia in both human and mouse, exons, introns, and the promoter region of the human Hyplip1 gene were sequenced in 61 Finnish FCHL families. We found 20 single nucleotide polymorphisms (SNPs): Ten in the promoter, one in an intron and nine in the last exon. None of the variants resulted in an amino acid change and all of them were relatively rare (allele frequencies < 0.1). Two-point and multipoint association analyses of the most common SNPs in 61 extended Finnish FCHL families revealed no evidence for association, indicating that the Hyplip1 gene is unlikely the causative FCHL gene. To analyze other regional candidate genes on 1q21-23, we genotyped 33 SNPs for 19 positional candidate genes in the region of the peak linkage markers, including apolipoprotein A2, upstream stimulatory factor 1, and retinoid X receptor gamma. Significant evidence for both linkage and association in Finnish FCHL families was obtained with some of the analyzed SNPs. We are currently analyzing these FCHL-associated SNPs in the representative population samples from Finland to identify the FCHL-associated variant.



**Analysis of the gene RAY1(FAM4A1/ST7)at a translocation breakpoint region on 7q31.3 in an autism patient reveals a complex multigene system.** *J.B. Vincent<sup>1,2</sup>, E. Petek<sup>3</sup>, S. Thevarkunnel<sup>1</sup>, J. Cheung<sup>1</sup>, S.W. Scherer<sup>1</sup>.* 1) Dept Genetics, Hosp Sick Children, Toronto, ON, Canada; 2) Dept. of Psychiatry, University of Toronto, ON, Canada; 3) Institute for Medical Biology and Human Genetics, University of Graz, Graz, Austria.

We recently identified a novel gene, RAY1 (FAM4A1), which spans a translocation breakpoint at 7q31.3 in a patient with autism. This gene has also recently been reported to be a suppressor of tumorigenicity, ST7. We have analysed this locus in more detail and revealed a multigene system that includes two novel non-coding anti-sense genes (RAYNC1 and RAYNC2) and a non-coding sense-strand gene (RAYNC3) that overlap with the coding RAY1 transcript. RAYNC1 overlaps with the RAY1 exon 1 and putative promoter and has several different polyadenylation sites. RAYNC2 spans from RAY1 intron 9 to intron 1, and has multiple isoforms. RAYNC3 spans from RAY1 intron 10 to exon 13 and includes RAY1 exons 11, 12 and 13, as well as at least six other intervening exons. Comparative sequence analysis between human and mouse genomic sequence at this locus has also revealed a number of intronic and intragenic regions that show a high degree of intra-species conservation. We have screened all exons of RAY1, RAYNC1, RAYNC2 and RAYNC3 for sequence variants in over 90 unrelated autism probands from multiplex families, and identified a number of polymorphisms and mutations. We have also identified a second autism patient with a chromosome 5:7 translocation mapping to the same cytogenetic band, 7q31.3, as the first patient. We have localized the breakpoint to a single BAC clone to which no genes have been mapped. We postulate that the non-coding genes around RAY1 are likely to be either cis-regulatory RNAs, particularly RAYNC1 which spans RAY1 promoter and exon 1, or may be evidence of promiscuous transcription, resulting in junk RNA.

**Identification of genes differentially expressed in wildtype and Fmr1 knockout mouse barrel cortex. W.T.**

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Fragile X syndrome is an X-linked dominant disorder caused by the absence of Fragile X Mental Retardation Protein (FMRP). Fmr1 knockout mice exhibit a cognitive and behavioral phenotype consistent with the human disorder, and show delayed dendritic spine maturation. Thus, at the cellular level, there is a difference between wildtype and knockout neurons. To investigate this difference *in vivo*, we sought to identify genes whose total expression levels are different between WT and KO mice. The following experiment was run in triplicate: the barrel cortices from 10 WT and 10 KO congenic c57BL/6 mice were dissected out during a critical period in neural development: P11-13. RNA was isolated from this tissue and used to probe oligonucleotide microarrays. Of the over 25,000 genes probed, 45% were present in the mouse barrel cortex. Four genes showed a substantial and consistent difference in expression between WT and KO in all three replicates. Real time, quantitative PCR was performed on three of these candidates and confirmed the change. We then used the available public databases to determine the identities of the differentially expressed genes: two of the genes are unknown ESTs (one overexpressed and one underexpressed in KO), the third is Fmr1, and the fourth is the beta chemokine exodus-2. Fmr1 expression is present in KO barrel cortex, but is much less abundant than in WT. This finding is not unexpected and provides an internal control for the experiment because of presumed nonsense mediated decay of Fmr1 knockout transcript. Exodus-2 is also less abundant in KO tissue. Though exodus-2 has not been shown to be involved in the developing mouse brain, recent findings have implicated cytokines in neural development.

**Variation in UCP3 is associated with multiple metabolic features in Caucasian females.** *C.M. Damcott<sup>1</sup>, E. Feingold<sup>1</sup>, S.P. Moffett<sup>1</sup>, J.A. Marshall<sup>2</sup>, R.H. Hamman<sup>2</sup>, J.M. Norris<sup>2</sup>, R.E. Ferrell<sup>1</sup>*. 1) Univ. of Pittsburgh; 2) Univ. of Colorado School of Med.

The uncoupling proteins (UCP) are a family of mitochondrial transport proteins that promote proton leakage across the inner mitochondrial membrane, uncoupling proton transfer from ATP production and releasing energy as heat. UCP3 is a member of this family uniquely expressed in skeletal muscle, a major tissue of thermogenesis. Gene expression studies in rodents suggest a role for UCP3 in energy metabolism, alterations in lipid, glucose, and insulin levels, and fuel substrate management. The UCP3 gene maps to human chromosome 11q13, a region linked to variation in obesity and REE. We tested the hypothesis that variation in the UCP3 gene is associated with aspects of obesity and/or diabetes mellitus in a sample of Caucasian females (n=223) from the San Luis Valley Diabetes Study. Resequencing of roughly 1kb 5' of exon 1 of UCP3 identified a C/T polymorphism at -55 bp. This variation was significantly associated with HOMA IR (p=0.01), a measure of insulin resistance, and marginally associated with triglycerides (TG; p=0.068). The C homozygotes have lower HOMA IR indexes (~3 units) and TG (~16 mg/dl). A second polymorphism in UCP3, a T/C silent substitution in codon 210 of exon 5, is also associated with HOMA IR (p=0.046) and TG (p=0.047). Presence of the C allele confers lower HOMA IR indexes (~3 units) and TG (~26 mg/dl), similar to C/C individuals at the -55 site. The linkage disequilibrium between these two variants suggests that the exon 5 C allele and the -55 C allele are marking a functional change in UCP3 affecting insulin resistance and TG levels. The exonic variant is also associated with fat mass measured by DEXA (p=0.002), percent body fat (p=0.018), and fasting free fatty acids (FFA; p=0.022). On average, T/T homozygotes exhibit a 5 kg higher fat mass, a 3% greater body fat, and increased FFA (+84 mol/l). Association of the exon 5 variant with these phenotypes, independent of the -55 variation, suggest the presence of additional functional variation in the UCP2/UCP3 region. This supports a role of UCP3 in regulating body weight, energy expenditure, lipid metabolism, and insulin action.

**Genotyping Single Nucleotide Polymorphisms in Four Different Amplicons in Diabetes.** *M.L. Robinson<sup>1</sup>, G. Wu<sup>1</sup>, S. Babu<sup>2</sup>, C. Roberts<sup>2</sup>, F. Bao<sup>2</sup>, P.R. Fain<sup>2</sup>.* 1) Applied Genomics and Molecular Genetics, Transgenomic Inc., Omaha, NE; 2) Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences, Denver, CO.

Single nucleotide polymorphisms (SNPs) are very useful markers for linkage disequilibrium studies aimed at the discovery of disease genes. Although a large number of SNPs have been identified and a high-density SNP map has been developed, cost effective methods for high-throughput SNP genotyping are lacking. As part of an effort to identify a susceptibility gene for type 1 diabetes, which maps to human chromosome 10q25.1, over 100 SNPs within a 125 kb interval at 10q25.1 were identified by direct sequencing of genomic DNA from 4 affected and unaffected family members. We designed and optimized primers to amplify 4 different amplicons which contain SNPs showing maximum linkage disequilibrium with the disease locus (IDDM17). Patients with known genotypes for each of the 4 SNPs were genotyped using a new primer extension protocol for the Transgenomic WAVE® DHPLC System. This is a rapid and inexpensive protocol suitable for high throughput genotyping.

**Epigenetic sequencing analysis of a H19-DMR reveals parent-of-origin independent methylation.** *T. Enklaar, M. Oswald, D. Prawitt, B. Zabel.* Children's Hospital , University of Mainz, Mainz, Germany.

Parent-of-origin specific DNA-methylation plays an important role in the process of controlling monoallelic expression of imprinted genes. For the human H19 gene two differentially methylated regions (DMRs) are known, residing around the promoter of the gene (DMR1) and in a region of 400bp direct repeats starting -2kb upstream of the H19 transcription start site (DMR2). They were frequently analyzed in order to find an association of a pathological methylation pattern with loss of imprinting implicated in diseases like Beckwith-Wiedemann-syndrome, Wilmstumour and colorectal cancer. The majority of previous investigations relied on the use of methylation-sensitive restriction enzymes like HpaII and SmaI detecting up to three restriction sites in each DMR. The sequencing of bisulfite treated DNA on the other hand reveals the methylation status of every single CpG-dinucleotide in the amplified region and allows to discriminate directly between the two parental alleles if single nucleotide polymorphisms are available. Thus, LOI of H19 in Wilmstumours and colorectal cancer was linked to hypermethylation of the DMR -2kb upstream of H19. Analysis of the H19 methylation status in Beckwith-Wiedemann-syndrome cases was up to now focused on the HpaII- and SmaI sites of the H19-promoter region revealing infrequent hypermethylation. We examined all involved CpG-dinucleotides of both differentially methylated regions in DNA of BWS patients and controls by bisulfite sequencing. Almost all CpGs near the H19 promoter, including the HpaII- and SmaI-sites showed parent-of-origin independent methylation. The degree of demethylation of the paternal allele as well as hypermethylation of the maternal allele was found to be highly variable. These studies suggest that silencing of the paternal H19 allele may not be directly related to methylation of DMR1 or seems to require only low levels of differential promoter methylation.

**Epigenetic differences between the parental alleles of *Ndn* (necdin), an imprinted gene in the Prader-Willi Syndrome region.** *M.L. Hanel, R. Wevrick.* Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Imprinted genes are expressed unequally on the two alleles depending on the parental origin. The neurobehavioural disorder Prader-Willi Syndrome (PWS) results from loss of the paternal complement of the chromosome region 15q11-q13 including *NDN* and other paternally expressed genes in this region. Mouse *Ndn* is located in the region of conserved synteny on chromosome 7 with the human PWS region. To investigate possible mechanisms for establishing and maintaining imprinting, we have studied *Ndn* for allele-specific methylation and chromatin conformation. We found that in brain, where necdin is expressed, the maternal allele is relatively hypermethylated compared with the paternal allele. In liver and heart, where necdin is not expressed, DNA methylation levels are low on both alleles. A distinctive but unstable maternal methylation pattern persists from the gametes until before the blastocyst stage (around implantation) and is then lost by the blastocyst stage where low levels of methylation are present on most paternal and maternal DNA strands. This suggests that for *Ndn*, DNA methylation may initially preserve a gametic imprint during preimplantation development, but that other epigenetic factors maintain the imprint later in embryonic development.

We are now analyzing the region for allele-specific differences in chromatin structure through DNase I sensitivity assays. Increased DNase I sensitivity is associated with an open chromatin conformation. In brain, the methylated, inactive maternal allele was more resistant to DNase I than the unmethylated, active paternal allele in the promoter region. In liver, both alleles were relatively resistant to digestion. Preliminary results in a search for allele-specific DNase I sensitivity outside the promoter region show that increased DNase I sensitivity on the paternal allele was detected at least 4 kb upstream of *Ndn*. In summary, allele-specific differences in DNA methylation and chromatin conformation are not evident in non-expressing tissues. However, methylation and chromatin conformation appear to be involved in allele-specific expression of *Ndn* in brain.

**Association between heteroplasmy of A3243G mutation and SNP in ND1 gene in 109 MELAS patients. J.**

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Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) is often associated with an A3243G mutation in tRNA-Leu(UUR) region of mtDNA. The patients with the mutation exhibit variable and multisystemic clinical manifestations, which are thought to be caused by different proportion (heteroplasmy) and distribution of the mutant mtDNA in the tissues. It is very important to know whether the mtDNA genome with A3243G mutation has a replicative advantage over wild-type one, because it may be closely related with the severity and the progression of the disease. In this study, we analyzed the single nucleotide polymorphism (SNP) other than A3243G mutation to know the possible involvement in the heteroplasmy of the mutant. We studied 109 DNA samples extracted from the muscles of MELAS patients carrying A3243G mutation. In order to quantify the mutation rapidly and accurately, we devised a real-time PCR amplification method using two fluorogenic probes in a single tube. The proportion of mutant mtDNA in these patients ranged from 7.5% to 100%. Then we selected 15 from 109 patients and divided them into two groups; one is high percentage level of the mutation group (10 patients; almost 100% of mutant DNA) and the other is low percentage level group (5 patients; from 7.5% to 43.6% of mutant DNA). We investigated the total mtDNA sequence of both groups and compared the SNPs. In result, total 194 SNPs were detected in both groups. We summarized these data in each group with radar chart divided into 16 functional units of mtDNA. The pattern of chart of 16S rRNA and ND1 gene appeared to be different between the groups. Then we screened the SNPs of the 16S rRNA and ND1 genes in all 109 patients. Several SNPs in ND1 gene were found to be accompanied with the high percentage level group more frequently in statistical significance. We conclude that the several SNPs in ND1 gene in mtDNA are related to the replicative advantage of mtDNA carrying A3243G.

**Pathogenic mechanism of mitochondrial tRNA<sup>Ser(UCN)</sup> 7511 mutation associated with non-syndromic deafness.**

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Hearing loss is one of the most common human sufferings, affecting one in 1000 children. Despite the recent progress in molecular characterization of deafness, the biochemical and molecular pathogenic mechanisms underlying the maternally inherited deafness remain poorly understood. Recently, an African-American family with maternally inherited nonsyndromic hearing loss has been found to be associated with the mitochondrial T7511C mutation in the tRNA<sup>Ser(UCN)</sup> gene, which is commonly related to deafness. In addition, 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. To understand the pathogenic mechanisms of these mtDNA mutations, a biochemical analysis has been carried out of transmitochondrial cell lines, constructed by transferring mitochondria from lymphoblastoid cell lines derived from deaf individuals with mtDNA mutations or from controls lacking mutations, into human mtDNA-less (r<sup>0</sup>) cells. A significant decrease in the amount of tRNA<sup>Ser(UCN)</sup> and rate of mitochondrial protein synthesis and oxygen consumption was observed in the five mutant cell lines, when compared with control cell lines. These observations suggest that the T7511C mutation in the tRNA<sup>Ser(UCN)</sup> gene is a primary mutation responsible for deafness phenotype and that T3308C and T5655C mutations play synergistic roles in the biochemical defect leading to deafness phenotype.



**DNA microarrays as a method to monitor changes in mitochondria-related gene expression in patients with OXPHOS defects and/or mitochondrial cardiomyopathy.** *J. Geurts<sup>1</sup>, B. van den Bosch<sup>1</sup>, L. Jacobs<sup>1</sup>, K. van der Kuijl<sup>1</sup>, S. van der Vlies<sup>1</sup>, I. de Coo<sup>2</sup>, H. Scholte<sup>2</sup>, J. Nijland<sup>2</sup>, T. Meitinger<sup>3</sup>, V. Tiranti<sup>4</sup>, M. Zeviani<sup>4</sup>, H. Smeets<sup>1</sup>.*

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One of the most important functions of mitochondria is the production of ATP by the process of oxidative phosphorylation (OXPHOS). OXPHOS defects become clinically manifest if the ATP production gets below a tissue-specific threshold level. Based upon energy consumption, the organs most likely to be affected are the central nervous system, skeletal and cardiac muscle, pancreatic islets, liver and kidney. In the case of the heart, OXPHOS defects may lead to hypertrophic, dilated or hypertrophic dilated cardiomyopathy, often as part of specific syndromes, but also as the sole expression of the defect. The 16 kb mitochondrial DNA encodes only 13 of the proteins involved in OXPHOS. The vast majority of mitochondrial proteins are encoded by the nuclear genome. To characterize the changes in expression of mitochondrial DNA- and nuclear DNA-encoded genes involved in the molecular pathogenesis of mitochondrial disease and mitochondrial cardiomyopathy in particular, we have developed a human mitochondrial DNA microarray encompassing approximately 600 mitochondria-related genes. This mitochondrial microarray is ideally suited for expression profiling experiments using patient-derived fibroblast cell lines and (cardiac) muscle biopsies. At this moment, biological material is available for different groups of patients with (1) complex I and/or complex I and IV deficiencies, (2) different mitochondrial mutations e.g MELAS, MERRF, NARP, Pearson syndrome, CPEO, LHON, Leigh syndrome and (3) mitochondrial cardiomyopathy. Results from initial experiments will be presented.

**The influence of mtDNA background on the disease process: a new primary Leber's Hereditary Optic Neuropathy mtDNA mutation requires European haplogroup J for expression.** *M.D. Brown<sup>1</sup>, O.A. Derbeneva<sup>2</sup>, Y. Starikovskaya<sup>2</sup>, J. Allen<sup>1</sup>, R.I. Sukernik<sup>2</sup>, D.C. Wallace<sup>1</sup>.* 1) Center for Molecular Medicine, Emory University School of Medicine, Atlanta, GA; 2) Institute of Cytology and Genetics, Russian Academy of Sciences, Novosibirsk, Siberia, Russia.

Lebers Hereditary Optic Neuropathy (LHON) is a form of blindness caused by mitochondrial DNA (mtDNA) mutations. Three mutations, 3460A, 11778A, and 14484C, account for 90% of LHON worldwide and are designated "primary" mutations. Primary mutations strongly predispose carriers to LHON, are not found in controls, are all in Complex I genes, and do not co-occur with each other. Previously, we demonstrated that the 11778A and 14484C mutations occurred more frequently than expected in association with European mtDNA haplogroup J (found in 9% of European-derived mtDNAs), suggesting a synergistic interaction among mtDNA mutations increased the probability of disease expression. Here, we report a new primary LHON mtDNA mutation, 10663C, affecting a Complex I gene. This mutation was homoplasmic in 3 Caucasian LHON families, all of which belonged to haplogroup J. These 3 families were the only haplogroup J-associated LHON families (out of 17) that did not harbor a known, primary LHON mutation. Comprehensive phylogenetic analysis of haplogroup J using complete mtDNA sequences demonstrated that the 10663C variant has arisen 3 independent times on this background. This mutation was not present in over 200 non-haplogroup J European controls, 74 haplogroup J patient and control mtDNAs, or 36 putative LHON patients without primary mutations. A partial Complex I defect was found in 10663C-containing lymphoblast and cybrid mitochondria. Thus, the 10663C mutation has occurred three independent times, each time on haplogroup J and only in LHON patients without a known LHON mutation. This makes the 10663C mutation unique among all pathogenic mtDNA mutations in that it appears to require the genetic background provided by haplogroup J for expression. These results provide further evidence for the predisposing role of haplogroup J and for the paradigm of "mild" mtDNA mutations interacting in an additive way to precipitate disease expression.

**Novel mtDNA C11777A Mutation in Leigh Syndrome Changes the Same Acid As G11778A Mutation in LHON.**

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Leigh syndrome (LS) is a heterogeneous disease, and defects in both mtDNA and nuclear DNA are reportedly involved in the pathogenesis of LS. However, in more than half of LS patients, the pathogenesis cannot be identified. A novel mtDNA C11777A mutation was identified in two unrelated patients out of 62 LS patients. Clinical symptoms including psychomotor retardation, abnormal signals on brain MRI, and hyperlactic acidemia were compatible with LS, but the clinical courses are relatively mild. Optic neuropathy was not detected. This mutation converted a highly evolutionary conserved arginine to a serine at codon 340 in the ND4 gene of complex I. The codon was also converted by a G11778A mutation, the most common mutation in LHON, but the amino acid replacement was different. This mutation existed in a heteroplasmic condition and did not detected 215 normal individuals and one patient's mother. No ragged-red or COX deficient fibers were detected by histopathologic studies of muscle biopsy. Spectrophotometric assays of skeletal muscle mitochondria showed significantly decreased activities in rotenone-sensitive NCCR with normal activities in SCCR and COX. ATP synthesis of myoblasts was significantly decreased when pyruvate/malate and glutamate/malate were used as substrates. ATP synthesis was normal when succinate and TMPD/ascorbate were used. These findings confirmed isolated complex I deficiency in their muscle. However, ATP synthesis of fibroblasts in one patient was normal, suggesting tissue-specific manner. It is noted that the phenotype of LHON patients is quite different from that the present cases despite the same amino acid substitution. These above findings indicate that this novel mutation caused a defect in energy production resulting in neuromuscular disease without optic neuropathy. Because the mean onset of LHON with 11778 mutation is their twenties, 11777 mutation may produce optic neuropathy in later life.

**Cloning and characterization of human *GTPBP3* gene, encoding a mitochondrial GTP binding protein.** X.M. Li, M.X. Guan. Human Genetics, Children's Hospital Med. Center, Cincinnati, OH.

*MSS1* in the yeast *Saccharomyces cerevisiae* encodes a mitochondrial GTP binding protein involved in the expression of *COX1* subunit of cytochrome c oxidase. *mss1* mutants manifest their respiratory-deficient phenotype only when coupled with a mitochondrial P<sub>R</sub><sup>454</sup> mutation, analogous to the deafness-linked A1555G mutation in the human mitochondrial 12S rRNA gene. Based on the available human EST sequences homologous to yeast *MSS1*, we have isolated full length cDNAs of human *GTPBP3* by use of SMART<sup>TM</sup> RACE cDNA amplification. *GTPBP3* gene (GeneBank accession no. AF360742) encodes a predicted protein with 493 amino acids, sharing a high identity to yeast *Mss1p*. This deduced protein contains the typical mitochondrial target sequence and three conserved domains: two GTP binding domains GTP1/OBG, and P21<sup>RAS</sup> as well as the SH3 domain, which is involved in protein-protein interaction. The coding region of this gene spans 8 exons, located at chromosome 19. Human *GTPBP3* gene is ubiquitously expressed as multiple transcripts. The ratio of different transcripts varies in several different tissue types. *GTPBP3*-GFP fusion experiment showed that *GTPBP3* is localized in mitochondrion. Additionally, the human *GTPBP3* can functionally complement the mutational phenotypes of yeast *mss1*. This suggested that human *GTPBP3* is the homolog of yeast *MSS1* and may be involved in the phenotypic expression of human deafness-linked A1555G mutation.

**Mitochondrial respiratory chain complex II mutations and the development of hereditary paragangliomas in the head and neck region.** *P.E. Taschner*<sup>1</sup>, *C.J. Cornelisse*<sup>2</sup>, *P. Devilee*<sup>1,2</sup>. 1) Dept Human/Clinical Genetics, Leiden Univ Medical Ctr, Leiden, Netherlands; 2) Dept Pathology, Leiden Univ Medical Ctr, Leiden, Netherlands.

Hereditary paragangliomas are usually benign slow-growing tumors, which arise from paraganglia, cell clusters of neuroectodermal origin. The most prominent paraganglion in the head and neck region is the carotid body, a chemoreceptive organ, which monitors arterial oxygen and maintains homeostasis. The occurrence of hereditary paragangliomas has been linked to at least three different loci: PGL1 on chromosome 11q22-q23, PGL2 on chromosome 11q13, and PGL3 on chromosome 1q21. Most families with hereditary paraganglioma are linked to the PGL1 locus. The inheritance pattern in these families is autosomal dominant with maternal imprinting. Recently, the PGL1 and PGL3 loci have been identified as the SDHD and SDHC genes, respectively, which encode the two membrane anchor proteins of mitochondrial respiratory chain complex II. Loss of the normal allele in paragangliomas suggests that the presence of these mitochondrial proteins is required to suppress tumor development. We hypothesize that mutations in the complex II membrane anchor genes result in abnormal oxygen sensing and interfere with normal development and maturation of the carotid body. Hyperplastic growth of the carotid body occurs, probably followed by additional somatic changes leading to monoclonal foci of tumor cells. The involvement of complex II in the development of paragangliomas provides evidence that mitochondria play a more important role in cellular oxygen sensing than suggested in current theories. This research is supported by the Dutch Cancer Society (KWF 98-1721).

**A Common mtDNA variant may be a susceptibility factor in 3 multifactorial diseases.** *J. Poulton<sup>1</sup>, V. Macaulay<sup>2</sup>, K. Livesey<sup>3</sup>, N. Wareham<sup>4</sup>, E. Parker<sup>1</sup>, D. Phillips<sup>5</sup>, D. Simmons<sup>6</sup>, B. Mayosi<sup>7</sup>, S. Khogali<sup>8</sup>, K. Robson<sup>3</sup>.* 1) University of Oxford Dept Paediatrics, John Radcliffe Hospital, Oxford OX3 9DU, UK; 2) University of Oxford Dept Statistics; 3) University of Oxford WIMM; 4) University of Cambridge; 5) MRC Environmental Epidemiology Unit, Southampton, UK; 6) University of Melbourne, Australia; 7) University of Oxford Dept Cardiovascular Medicine; 8) University of Birmingham, UK.

Type 2 diabetes and dilated cardiomyopathy (DCM) are important multifactorial conditions. Haemochromatosis is an autosomal recessive disorder with incomplete penetrance. Diabetes, cardiomyopathy and liver failure are all features of mitochondrial disease. Hence mtDNA variants may play a role in type 2 diabetes, DCM and haemochromatosis. The common 16189 variant lies near to control sequences in human mtDNA. **Diabetes Studies** 1. *Case control study* in 417 European diabetics and 427 controls. 16189 variant increased in diabetics, OR=1.67(p=0.037, 95%CI 1.0-2.6). 11/11 individuals with both paternal family history of diabetes and variant were diabetics OR= . 2. *Hyperinsulinaemia* associated with: the 16189 variant in 2 European populations (p=0.015) length variation due to 16189 variant among 190 Polynesians (p=0.008). 3. *16189 variant & thinness*: associated with: Decreased Ponderal Index at birth in 'changers' with the 16189 variant (p=0.007) in a birth cohort of 885. High placental to birth weight ratio, acute insulin response & glucose effectiveness in an Australian birth cohort of 160 individuals (p=0.025, 0.022, 0.005) **DCM study** The 16189 variant is associated with increased risk of DCM in Caucasians & Africans (p=0.01; OR=2.15 [95% CI 1.16-3.98] and p=0.036 OR=4.51 (95%CI 1.03-19.65, ) respectively). **Haemochromatosis study** The 16189 variant is present in 19/113 (17.9%) of individuals expressing the haemochromatosis phenotype who are homozygous for the C282Y allele compared with 8.8% of controls (p=0.01). The 16189 variant may influence penetrance. The 16189 variant per se may subtly affect mitochondrial function as 1) founder effect excluded 2) associations between 4 phenotypes in a total of 8 populations.

**Rescue of the frataxin knockout mouse embryonic lethal phenotype by human YAC and BAC transgenesis.** *M. Pook<sup>1</sup>, S. Al-Mahdawi<sup>1</sup>, C. Carroll<sup>1</sup>, M. Cossee<sup>2</sup>, H. Puccio<sup>2</sup>, L. Lawrence<sup>1</sup>, P. Clark<sup>1</sup>, M. Lowrie<sup>1</sup>, J. Bradley<sup>3</sup>, J.M. Cooper<sup>3</sup>, M. Koenig<sup>2</sup>, S. Chamberlain<sup>1</sup>.* 1) Cell & Molecular Biology, Leukocyte Biology and Biological Structure & Function, BMS, Imperial College School of Medicine, London, UK; 2) IGBMC, CNRS/INSERM Universit Louis Pasteur, Hopitaux Universitaires de Strasbourg, 67404 Illkirch, France; 3) University Dept. of Clinical Neurosciences, Royal Free and University College Medical School, London, UK.

Friedreichs ataxia (FRDA) is an autosomal recessive progressive neurodegenerative disorder caused by decreased expression of the nuclear encoded mitochondrial protein, frataxin. Previous studies have suggested involvement of mitochondrial iron accumulation and iron-sulphur enzyme deficiencies in FRDA pathogenesis, and embryonic lethality of homozygous *Frda* knockout mice has indicated a role for frataxin in development. To study FRDA pathogenesis in more detail, mouse models that demonstrate reduced levels of frataxin function are required. As an initial test for the feasibility of re-introducing functional human frataxin into a null mouse, we have established three YAC and two BAC wild-type human FRDA transgenic lines, containing different amounts of human genomic DNA (140kb to 370kb) flanking an intact FRDA gene. To date, one YAC (370kb) and both BAC (140kb and 180kb) transgenic lines have been crossed with heterozygous *Frda* knockout mice. Phenotypically normal offspring that express only human frataxin, and no endogenous mouse frataxin, have been identified from all three lines. The human frataxin is expressed in the appropriate tissues at levels comparable to the endogenous mouse frataxin, and it is correctly processed and localised to mitochondria. Biochemical analysis of heart tissue shows preservation of mitochondrial respiratory chain function. Thus, we have demonstrated that human frataxin can effectively substitute for endogenous murine frataxin in the null mutant. These studies are of immediate consequence for the generation of FRDA transgenic mouse models and further contribute to the accumulating knowledge of human-mouse functional gene replacement systems.

**Gene expression alterations in tau-deficient mice.** *F. Oyama*<sup>1,2</sup>, *S.E. Kotliarova*<sup>2</sup>, *A. Harada*<sup>3</sup>, *M. Ito*<sup>4</sup>, *Y. Ueyama*<sup>4,5</sup>, *N. Hirokawa*<sup>3</sup>, *N. Nukina*<sup>2</sup>, *Y. Ihara*<sup>1,6</sup>. 1) Neuropathology, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 2) Molecular Neuropathology Group, RIKEN Brain Science Institute, Saitama, Japan; 3) Cell Biology and Anatomy, Grad Sch Med, Univ Tokyo, Tokyo, Japan; 4) Central Institute for Experimental Animals, Kanagawa, Japan; 5) Pathology, Sch Med, Tokai Univ, Kanagawa, Japan; 6) CREST, Saitama, Japan.

Tau, a microtubule-associated phosphoprotein, plays an important role in determining and maintaining neuronal morphology. Its hyperphosphorylated form is the major constituent of paired helical filaments (PHF) in Alzheimers disease (AD). Recently a number of exonic and intronic mutations in the tau gene were identified in the patients affected by frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). This disease is characterized by extensive neuronal loss in certain regions associated with PHF-like fibril formation. Thus, this is definite genetic evidence that tau can kill the neuron. To find an as yet unidentified role of tau in vivo, we used oligonucleotide DNA arrays profiling ~11000 mRNAs to see how the gene expression is altered in the brain from tau-deficient mice at 1 and 8 weeks of age, compared with control mice at the same ages. This microarray screening suggested that 19 and 74 genes are significantly changed at 1 and 8 week aged tau-deficient mice, respectively. To confirm the data by the microarray we performed quantitative RT PCR or ribonuclease protection assay. We found that the levels of mRNAs encoding the transcription factors, inflammatory proteins, cytokines, chemokines and growth factors are markedly increased at 8 weeks of age in the brain of tau deficient mice, while the expressions of suggested genes were not significantly altered at 1 week of age. These results indicate that tau-deficiency directly or indirectly leads to overexpression of a distinct set of genes involved in transcription and inflammation at 8 weeks of age.



**Characterization of the Kelch-like 1 (KLHL1) protein.** *Y. He, J. Nemes, M.D. Koob.* Inst Human Genetics, U of MN, Minneapolis, MN.

The KLHL1 protein is encoded by the sense mRNA of the sense/antisense transcriptional unit at the spinocerebellar ataxia type 8 (SCA8) locus on chromosome 13. We have previously reported that both the KLHL1 sense and antisense transcripts are evolutionarily conserved and are primarily expressed in brain tissues in both mouse and man. We have now begun characterization of the KLHL1 protein to determine what function this protein serves, with the ultimate goal of determining if disruption of KLHL1 function plays a role in the neuropathology of SCA8. Based on our results to date, we are hypothesizing that KLHL1 is an actin-organizing protein that is involved in either establishing or maintaining portions of the actin-cytoskeleton of the neurons in which it is expressed. Analysis of the predicted amino acid sequence of KLHL1 indicates that it contains a POZ protein-protein interaction domain that may allow KLHL1 to multimerize, and a potential actin-binding domain consisting of six KELCH repeat units. In order to experimentally determine if KLHL1 has these predicted properties, we over-expressed and purified the amino-terminal fragment of KLHL1 containing the POZ domain using an *E. coli* expression system. We found that this protein fragment does, as expected, form multimers with itself. We were also able to demonstrate that KLHL1 binds actin *in vivo* by co-immunoprecipitating actin from mouse brain protein-extracts using a purified rabbit polyclonal antibody raised against this KLHL1 fragment. Preliminary immunohistochemistry using the KLHL1 polyclonal antibody and a primary culture of mouse granule cells indicates that KLHL1 is primarily localized to actin-rich neuronal structures (e.g., growth cones and synaptic junctions). We are continuing to confirm and expand these cellular and subcellular localization studies using newly developed KLHL1 monoclonal antibodies.

**Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy (DM) types 1 and 2.** *C.A. Thornton<sup>1</sup>, A. Mankodi<sup>1</sup>, C. Urbinati<sup>2</sup>, R. Moxley<sup>1</sup>, V. Sansone<sup>1</sup>, M. Swanson<sup>2</sup>.* 1) Univ Rochester, Rochester, NY; 2) Univ Florida, Gainesville, FL.

There is locus heterogeneity in DM. DM1 is caused by expansion of a CTG repeat in the DMPK gene on chr. 19. DM2 is linked to chr. 3. Pathogenic effects in DM1 are likely mediated, at least in part, by the expanded CUG repeat in mutant mRNA. The mutant transcripts are retained in the nucleus in multiple foci. Various proteins interact with CUG repeats in vitro, but none have been shown to interact in vivo. We investigated the possibility that DM2 is caused by expression of a CUG repeat or related sequence. RNase protection with a CAG-29 probe showed an expanded CUG repeat in DM1 but not in DM2 or normal controls (n=4 in each group, myoblast RNA). However, FISH using CAG probes on sections of muscle tissue showed nuclear foci in DM2 similar to those in DM1. Nuclear foci were present in muscle tissue from all patients with symptomatic DM1 (n=9) or DM2 (n=9) but not in disease or healthy controls (n=23). Foci were not seen with CUG- or GUC-repeat probes. Foci in DM2 were distinguished from DM1 by lower stability of the probe-target duplex, suggesting that a sequence related to CUG repeats accumulates in the DM2 nucleus. Furthermore, muscleblind, homologue of a protein required for muscle development in *Drosophila*, localized to the nuclear foci in both DM1 and DM2. In contrast to previous studies showing hundreds of RNA foci per nucleus in DM1 myoblasts, we found 1 to 3 foci per nucleus in muscle tissue from DM1 and DM2 patients. The foci did not associate with a specific nuclear structure, as determined by markers for nucleoli, coiled bodies, PML bodies, or perinucleolar complex. To identify proteins that interact with expanded CUG repeats in vivo, we examined muscle from transgenic mice that express expanded CUG repeats. Among 9 proteins examined, including 7 dsRNA binding proteins and CUGBP1, only muscleblind colocalized with foci of expanded CUG repeats. These results support the idea that nuclear accumulation of mutant mRNA is pathogenic in DM1, suggest that a similar disease process occurs in DM2, and point to a role for muscleblind in the pathogenesis of both disorders.

**Genotyping microarray (gene chip) for the *ABCR* (*ABCA4*) gene.** R. Allikmets<sup>1</sup>, A. Hutchinson<sup>1</sup>, K. Jaakson<sup>2</sup>, M. Külm<sup>2</sup>, H. Pavel<sup>2</sup>. 1) Dept Ophthalmology, Columbia Univ, New York, NY; 2) Asper, Ltd., Tartu, Estonia.

Variants in the *ABCR* (*ABCA4*) gene have been associated with at least five distinct macular degeneration phenotypes, including Stargardt disease (STGD), cone-rod dystrophy (CRD), and age-related macular degeneration (AMD). Comparative genetic analyses of *ABCR* variation have been complicated due to high allelic heterogeneity of the gene and differences in screening methods. To overcome these limitations, we designed a genotyping microarray (gene chip) for *ABCR*, which includes all >320 variants currently described in this gene, allowing for the detection of all known *ABCR* variants in a DNA sample in one simple reaction. The ABCR320 chip was constructed by the allele-specific primer extension (APEX) technology (described at: <http://www.asper.ee>). Every sequence change described in the *ABCR* gene was included on the chip by synthesis and application of allele-specific oligonucleotides. Screening of 150 STGD patients and 100 controls, previously analyzed by us with the SSCP technology, was performed to validate the ABCR320 chip. The microarray yielded >90% effective in determining the existing genetic variation, and proved to be comparable to direct sequencing in that it yielded many sequence changes that had gone undetected by SSCP. Moreover, the cost of screening was below 25 cents per genotype, which is at least five times less expensive than similar service currently available by any comparable technique and/or provider. The ABCR320 genotyping microarray represents the most cost-effective and comprehensive screening tool for genetic variation in one gene. This is the first instance where a gene chip is made available for the genotyping of patients with eye disease(s), including STGD/FFM, CRD, RP, and AMD. Latest results from screening patients with these phenotypes will be presented at the meeting. The ABCR chip serves as a prototype for the next generation of screening and diagnostic tools in ophthalmic genetics, and will serve as a bridge between clinical and scientific research.

**Haplotype Analysis of b-Thalassemia in Iran.** *N. Ghobadi*<sup>1</sup>, *T. Parsa*<sup>1</sup>, *B. Moghimi*<sup>2</sup>, *A.R. Noorian*<sup>2</sup>, *S. Teimourian*<sup>3</sup>, *H. Najmabadi*<sup>4</sup>. 1) Department of Genetics, University of Welfare & Rehabilitation, Evin, Tehran, Tehran, Iran; 2) Tehran University of Medical Sciences, School of Medicine, Tehran, Iran; 3) Institute of Biochemistry and Biophysics (IBB), Tehran University of Sciences, Tehran, Iran; 4) Dept Molecular Biol, Torrance, CA.

Beta thalassemia is the most common genetic disorder in Iran. Over 25 mutations have been identified so far. The objective of this study is to perform haplotype analysis of these mutations in order to determine their origin and historical background. So far we have studied 34 b-thalassemia individuals with confirmed mutations IVSII-1, C44, C8, C22 and FR36-37. We constructed a related haplotype for each mutation type in six polymorphic loci (Xmn 1, Hind III G/g, Hind II/3`wb, Rsa 1/b, Ava II/b and Hinf 1/b). Our results indicate that majority of individuals with IVS II-1, C8, C22 and FR36-37 mutations have two haplotypes while C44 mutation showed one haplotype.

**Variant Analysis of the ABCR (ABCA4) Gene in an AMD Patient Population.** *J.R. Gilbert<sup>1</sup>, S. Schmidt<sup>1</sup>, E. Postel<sup>1</sup>, C. Allen<sup>1</sup>, W. Scott<sup>1</sup>, A. Agrawal<sup>2</sup>, M. Hogan<sup>2</sup>, J. Haines<sup>2</sup>, M.A. Pericak-Vance<sup>1</sup>.* 1) Dept Medicine, Ctr Human Gen, Duke Univ, Durham, NC; 2) Program in Human Genetics, Vanderbilt Univ, Nashville, TN.

Age-related macular degeneration (AMD) is the most common cause of severe vision loss among individuals over age 50 in the U.S. The etiology of this devastating disease is unknown. Evidence implicates a combination of genetic, environmental, and biological factors in the pathogenesis of AMD. Variants in the gene encoding ABCR (ABCA4), a photoreceptor-specific ATP-binding cassette transporter, have been implicated in the etiology of AMD in some patients and families. We have begun a complete analysis of the ABCA4 gene in a large cohort of AMD families and age and sex matched controls. Two clinical subtypes of advanced AMD have been described and are referred to as an exudative (wet) form characterized by choroidal neovascularization (CNV) and a dry form characterized by geographic atrophy. Vision loss may be severe with either form. Individuals were considered unaffected if they were classified as grade 1 (no or small (<63  $\mu$ m) drusen) or grade 2 (non-extensive intermediate (>63  $\mu$ m) drusen). Individuals with extensive intermediate or any large (>125  $\mu$ m) soft drusen were classified as grade 3 and considered affected. Similarly, those with the more advanced findings of geographic atrophy (grade 4) or exudative lesions (grade 5) were considered to suffer from AMD. Initial analysis has begun with 194 individuals from 67 families. These individuals were grouped into 45 pools for rapid processing. All 50 exons of the ABCR gene including the promoter region have been subjected to mutation screening with denaturing high-performance liquid chromatography (DHPLC). Variants have been detected in multiple families for exons 3, 6, 12, 25, 29, 33, 41, 44, and 50. Variants have been detected in single families for exons 8, 30, 37 and other exons. Many families show multiple changes within the same family. A full cataloging of variants in all exons and the promoter of ABCA4 in these families will be presented.

**Gene-based screening for Y-chromosome deletions in Taiwanese men presenting with spermatogenesis failure.** *P. Kuo*. Dept OB/GYN, National Cheng-Kung Univ Hosp, Taiwan, Republic of China.

**Objective:** To develop a protocol for gene-based screening of Y chromosome deletions in men with spermatogenic failure. **Materials and Methods:** Two hundred and two infertile men presenting with severe oligozoospermia and non-obstructive azoospermia. Fifteen gene-specific markers were applied for screening of Y chromosome deletion status. Three genes are located in the AZFa region: DFFRY, DBY, and UTY. Four genes are located in the AZFb region: EIF1AY, PRY (multiple copies, also s in 4A and 6E), TTY2 (multiple copies, also in 4A) and RBM1. Three genes are located in the AZFc region: DAZ, BPY2, and CDY1. We also screened 5 genes beyond AZF region: TB4Y, BPY1, CDY2, XKRY and SMCY. A multiplex polymerase chain reaction (PCR) amplification system was developed to facilitate rapid screening. Another set of 24 STS markers was also used in all patients in order to define the position and extent of deletion. This part of experiment included 13 markers from interval 5 (sY81, sY82, sY84, sY88, sY182, sY94, sY95, sY97, sY102, sY105, sY109, sY 117, and sY127) and 11 markers from interval 6 (sY 143, sY164, sY134, sY138, RBM1 gene, sY147, sY149, sY153, sY 277, sY283, SPGY). **Result(s):** Of 180 patients evaluated, 19 were found to harbor deletions of one or more genes including DFFRY, DBY, RBM1, DAZ, CDY1 and BPY2. The deletion frequency in this series was 10.6%. Only 16 out of 19 gene-deleted patients were found to have deletions by the second round of STS screening. The 3 patients (case 1-3) in whom the deletion failed to be detected by STS markers harbor deletions of DFFRY and DBY gene, respectively. Except for these three cases, the positions and extents of deletion interval of 16 Y-deleted patients were compatible between the 2 methods. **Conclusion:** Gene-based multiplex PCR screening provides a rational alternative to detect deletion status of Y chromosomal genes in infertile men. As compared with approach based on anonymous STS markers, it provides more rational tool for genotype/phenotype correlations.

**Mutation analysis of UBE3A gene in Angelman syndrome patients.** *G.A. Molfetta*<sup>1,2</sup>, *W.A. Silva-Jr*<sup>3</sup>, *J. Wagstaff*<sup>2</sup>, *J.M. Pina-Neto*<sup>1</sup>. 1) Dept Genet, Sch Medicine, Ribeirao Preto - USP, Brazil; 2) Dept Biochem Molec Genet, Sch Medicine, University of Virginia, USA; 3) Molec Biol Lab, Cellular Therapy Center, Blood Center, Sch Medicine, Ribeirao Preto - USP, Brazil.

Angelman syndrome (AS) is a clinical condition characterized mainly by congenital hypotonia, delayed neuropsychomotor development, severe mental deficiency, loss of speech, bursts of inappropriate laughter, seizures and ataxia. Different genetic mechanisms can result in loss of expression of the gene responsible for AS: 70% are maternal 15q11-13 deletions; 7-9% are imprinting defect at the 15q11-13 region; 3-5% show paternal uniparental disomy (UPD); 4-8% present mutations in the UBE3A gene and 10-15% have no deletion, imprinting defect, UPD or UBE3A mutation. The UBE3A gene (E6-AP ubiquitin-protein ligase) is the elected gene for AS because it suffers tissue-specific imprinting in brain tissue and some point mutations in this gene lead to AS. The aim of this work was to screen for mutations in the UBE3A gene in 63 patients suspected of having AS with normal molecular analysis. We used the SSCP technique to screen for mutations in all the 16 exons from the UBE3A gene. When an abnormal shift was found the DNA sequencing was carried out and the sequences were compared with GenBank access U84404. We found that 5 cases showed abnormal shifts by SSCP. Performing the DNA sequencing we found that 3 cases had sequence variants inherited from the father and therefore not pathogenic: 1118G®A (A178T), 1003A®T (A139A) and 3UTR del114bp, the last one having already been reported. One case showed a T®C at position 9 of the intron 12 and another case showed a 3UTR dup4bp. Parental samples of this last patient were not available but we believe this is not a mutation because the abnormality is located at the 3UTR region, which has already been shown to have only benign variants. We concluded that the molecular approach here described is useful to detect abnormalities in the UBE3A gene. We would like to reinforce the use of this methodology associated with a more stringent clinical criteria, in order to better select patients that would be submitted to this molecular investigation. Supported by: FAEPA, FAPESP.

**Mutational analysis of AIRE gene in APS2 patients.** *R. Mango<sup>1</sup>, S. Guarino<sup>1</sup>, R. D'Apice<sup>1</sup>, F. Amati<sup>1</sup>, F. Sangiuolo<sup>1</sup>, P. Pozzilli<sup>2</sup>, G. Bottazzo<sup>3</sup>, A. Crinò<sup>3</sup>, R. Buzzetti<sup>4</sup>, G. Novelli<sup>1</sup>.* 1) Dept. of Biopathology, Tor Vergata University, Rome, Roma, Italy; 2) Dept. of Internal Medicine, Tor Vergata University, Rome, Italy; 3) Ospedale Bambino Ges, Rome, Italy; 4) Dept. of Internal Medicine, La Sapienza University, Rome, Italy.

Autoimmune polyendocrine syndrome, type II; (APS2, OMIM 269200), is characterized by adult onset adrenal failure associated with type I diabetes and/or hyperthyroidism. APS2 is believed to be polygenic, characterised by dominant inheritance and association with HLA DR3. In the effort to identify genes involved in this syndrome, we decided to perform a mutational analysis of the coding region the autoimmune regulator (AIRE) gene in a group of 29 patients. AIRE gene represents the causative gene for APECED (autoimmune polyendocrinopathy-candidiasis ectodermal dystrophy) or APS1 (OMIM 240300). All fourteen exons of AIRE gene, were amplified from genomic DNA with primers located in respective flanking introns and analysed with DHPLC. We detected an heteroduplex peak in a patient, the following sequencing reaction showed a C®T transition at 717nt that resulted in a silent mutation. However this mutation was absent in a group of 50 controls. The same patient showed a C®A transversion in intron 7 (IVS7 41 C®A). An additional polymorphism was detected in intron 10 (IVS10 5G®A) in three patients. This variant was also detected in the control group. These results rule out the role of AIRE gene in etiology of autoimmune polyendocrine syndrome type II.



**The Arginine to Tryptophan Mutation in Codon 394 of the Wilms' Tumor Gene (R394W), a Mutation Hotspot for Denys-Drash (DDS) and Frasier syndromes (FS), is Uncommon in Primary Sporadic Focal Segmental Glomerulosclerosis (FSGS).** *S.M. Orloff<sup>1</sup>, S.K. Iyengar<sup>1</sup>, W. Briggs<sup>3</sup>, R. Dart<sup>3</sup>, S. Kobert<sup>3</sup>, M. Mokryzcki<sup>3</sup>, D. Vlahov<sup>3</sup>, P. Kimmel<sup>3</sup>, T. Ahuja<sup>3</sup>, J. Berns<sup>3</sup>, E. Simon<sup>3</sup>, M. Smith<sup>3</sup>, H. Trachtman<sup>3</sup>, M. Michel<sup>3</sup>, C. Winkler<sup>2</sup>, J.R. Schelling<sup>1</sup>, J.R. Sedor<sup>1</sup>, J.B. Kopp<sup>2</sup>.* 1) Epidemiology and Biostatistics and Medicine, Case Western Reserve Univ, Cleveland, OH; 2) NIH-NIDDK, Bethesda, MD; 3) Other.

Heterozygous mutations in the Wilms' tumor (WT1) gene are associated with early onset, diffuse mesangial sclerosis (DMS) and FSGS as part of DDS and FS. Based on evidence from rare DDS and FS patients, we hypothesized that WT1 mutations occur in sporadic, more common, forms of glomerular scarring, and confer risk to progressive nephropathy. To test our hypothesis, patients with primary FSGS enrolled in a multi-center study were screened for the R394W WT1 mutation, which functions in trans to inhibit wild type WT1 function. Our study is largest mutation analysis conducted to determine if variants at WT1 cause idiopathic FSGS. Cases, recruited at 13 US Medical centers, were African American (AA) (62F, 109M), Caucasian (15F, 29M), Hispanic (4F, 6M) or Asian (3F) with primary renal disease (idiopathic, heroin, HIV and collapsing FSGS). AA controls (143M, 50F) were HIV seropositive, with HIV duration > 8 years, with no clinical evidence of renal disease as demonstrated by serum creatinine < 1.5mg/dl and urine protein/creatinine < 0.05. The 224 base pair (bp) exon 9 product was digested with Hpa II. Hpa II digested, wild type, arginine-containing, alleles generate fragments of 118 and 106 bps, while the mutation-bearing alleles were undigested. We found only one case and one control that carried the homozygous R394W mutation, and no heterozygotes. We conclude that the WT1 R394W mutation is not common in sporadic FSGS and the presence of this mutation in a control suggests it is insufficient to cause disease. Further mutation analyses of WT1 may identify nephropathy risk alleles.

**Alpha 1- antichymotrypsin gene analysis in allergic asthmatic children.** *C. Quinzii, C. Bombieri, G. Malerba, P.F. Pignatti.* Section of Biology & Genetics, DMIBG, University of Verona, Verona, Italy.

Asthma is a complex disease, phenotypically heterogeneous, associated with intermittent respiratory symptoms, bronchial hyperresponsiveness (BHR), atopy and reversible airflow obstruction. This disease results from exposure to environmental factors in genetically susceptible individuals. Linkage analysis and transmission disequilibrium test on chromosome 14 markers in 189 families (847 individuals) with asthmatic children suggested that alpha 1-antichymotrypsin gene (AACT) or a closely located gene may be involved in susceptibility to allergic asthma, in particular to BHR (*J Allergy Clin Immunol* 2000; 107:654). We now report an analysis for AACT gene mutations in a sample of 19 asthmatic children from North East Italy, characterized for atopy, hyperresponsiveness to metacholine, total serum IgE levels, skin prik test (SPT), and clinical asthma, and selected for the presence of BHR positivity among the above cited patients, and in 18 healthy controls tested for the same phenotypes. Denaturing gradient gel electrophoresis (DGGE) was performed for exons 1, 3, 4 and 5 and their intronic flanking regions; direct sequencing is being performed for exon 2. DGGE screening showed the presence of a mutation in 1 asthmatic patient and in 2 control subjects, respectively. At the moment only one of the mutations is characterized: a newly described mutation 834 A@C in exon 3 (Ala 249 Ala), of one control subject. Sequencing of the other two mutations, and of exon 2 is proceeding. Presently available data do not indicate an association between alpha 1-antichymotrypsin mutations and allergic asthma in addition to the Thr-15Ala polymorphism previously reported.

**Increased noise as an effect of haploinsufficiency of the tumor suppressor gene Neurofibromatosis type 1 in vitro.**

*D. Kaufmann*<sup>1</sup>, *S. Schrank*<sup>2</sup>, *H. Gruler*<sup>2</sup>, *W. Vogel*<sup>1</sup>, *R. Kemkemer*<sup>2</sup>. 1) Dept of Human Genetics, University of Ulm, D 89070 Ulm, Germany; 2) Dept of Biophysics, University of Ulm, D 89070 Ulm, Germany.

In human diseases related to tumor suppressor genes, it is suggested that only the complete loss of the protein results in specific symptoms such as tumor formation, whereas simple reduction of protein quantity leading to haploinsufficiency essentially does not affect cellular behavior. Using a model of gene expression it was presumed that haploinsufficiency is related to an increased noise in gene expression also in vivo (Cook et al.1998). Here, we demonstrate that haploinsufficiency of the tumor suppressor gene Neurofibromatosis type 1 (NF1) results in an increased variation of dendrite formation in cultured NF1 melanocytes. These morphological differences between NF1 and control melanocytes can be described by a mathematical model where the cell is considered as a self-organized automaton. The model describes the adjustment of the cells to a set point and introduces some noise into that process. It fits the experimental data of control and NF1 melanocytes. In the cells haploinsufficient for NF1 we found an altered signal-to-noise ratio detectable as increased variation in dendrite formation in 2 out of 3 investigated morphological parameters. We also suggest that in vivo NF1 haploinsufficiency results in an increased noise in a cellular regulation and that this effect of haploinsufficiency might be found also in other tumor suppressors.

**A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression.** *M.A. Kennedy<sup>1</sup>, R.L. Roberts<sup>1</sup>, R.T. Mulder<sup>2</sup>, P.R. Joyce<sup>2</sup>*. 1) Department of Pathology, Christchurch School of Medicine, University of Otago, Christchurch, New Zealand; 2) Department of Psychological Medicine, Christchurch School of Medicine, University of Otago, Christchurch, New Zealand.

The human multi-drug resistance gene *ABCB1* encodes the P-glycoprotein (P-gp) that regulates movement of many substances across the blood-brain barrier. Evidence from a knockout mouse lacking P-gp (Uhr et al. 2000; *Neuropsychopharmacology* 22,380-387) suggests that the TCA amitriptyline and its metabolites are substrates for P-gp. In these mice penetration of amitriptyline, but not the SSRI fluoxetine, into the brain is enhanced. We reasoned that polymorphisms of P-gp may affect responses of patients to antidepressant drugs. A polymorphism of *ABCB1* (3435C>T) was recently correlated with expression levels and in vivo function of P-gp (Hoffmeyer et al. 2000; *PNAS* 97,3473-3478). We developed a DNA test for this SNP, and genotyped 165 Caucasian patients with major depression enrolled in a randomized antidepressant treatment trial of nortriptyline and fluoxetine. We observed a significant association between nortriptyline-induced postural hypotension (NIPH) and 3435C>T (chi-square = 6.75, df = 2.73, p=0.034). Of nortriptyline-treated patients, 25% (4 of 16) who were TT and 7% (3 of 43) who were heterozygous suffered symptomatic NIPH. None of the 17 patients who were CC and no fluoxetine-treated patients experienced postural hypotension. Our results suggest that presence of one or more T alleles at the 3435C>T polymorphism of *ABCB1* is a risk factor for occurrence of NIPH.

**Investigation of relation of NOD2 mutations to treatment response to infliximab and clinical course of Crohn's Disease.** *J. Hampe<sup>1</sup>, S. Mascheretti<sup>1</sup>, J. Grebe<sup>1</sup>, P.J.P. Croucher<sup>1</sup>, K. Huse<sup>2</sup>, H. Frenzel<sup>1</sup>, S. Nikolaus<sup>1</sup>, S. Schreiber<sup>1</sup>.* 1) General Internal Medicine, University Kiel, Kiel, Germany; 2) Institute for Molecular Biotechnology Jena, Germany.

Genetic predisposition to inflammatory bowel disease (IBD) has been demonstrated by epidemiological and linkage studies. Genetic linkage of IBD to chromosome 16 has been previously observed and replicated in independent populations. Recently, NOD2 has been identified as the first susceptibility gene for Crohn's Disease on chromosome 16 in four different populations. NOD2 has a strong influence on the activation of NFkB.

We investigated the influence of three mutations in the leucine-rich region of the NOD2 gene on treatment response to infliximab (Anti-TNF-alpha antibody) in a cohort of 93 patients treated in a controlled clinical trial. The influence of NOD2 variants on the natural course of disease was investigated in 180 prospectively and 200 retrospectively characterized German Crohn's Disease patients.

No significant association of NOD2 variants with the response to infliximab treatment was found. Further investigation is needed to define the influence of NOD2 variation of treatment response and clinical course of Crohn's Disease.

**MAPPING OF GENES IN THE AUTISTIC DISORDER REGION ON CHROMOSOME 15q11-q13.** *S.J. Kim, M.M. Menold, J. Stajich, M.A. Pericak-Vance, J.R. Gilbert.* Department of Medicine and Center for Human Genetics, Duke University Medical Center, Durham, NC 27710.

Chromosome 15q11-q13 has been implicated in the genetic etiology of autistic disorder (AutD). To identify candidate AutD genes, a physical contig map was generated from the GABRB3 receptor to the OCA2 gene. To characterize AutD candidate genes within the genomic contig, 28 BAC, PAC and P1 clones containing numerous rare restriction sites were analyzed using Island Rescue PCR (IR-PCR). 150 EagI, BssHII and SacII related CpG island sites were cloned, sequenced and analyzed. BAC/PAC sequencing and multiple comparison analysis have identified 83 unique clones. All IR-PCR clones had the characteristics of 5' end of gene or promoter sequence. 45 unique CpG islands met full CpG island criteria. 31 CpG island clones were mapped onto the human genomic draft contigs spanning the region from GABRB3 gene to the APBA2 gene. 14 CpG clones including GABRG3 and APBA2 showed expression in human fetal brain tissue. mRNA homology searching results performed by BLAST analysis for 31 CpG island clones included known GABRG3 gene, APBA2 gene and E1-E2 ATPase gene as well as 16 ESTs. 13 IR-PCR clones were very close to CpG island criteria. 25 IR-PCR clones did not meet CpG island criteria. 13 clones showed expression in human fetal brain tissue. 9 clones showed a homology to ESTs. Transcript map in this region was generated with CpG island gene markers along the contig. Five known genes including GABAA receptor subunits, APBA2, and numerous ESTs colocalized with CpG islands in this region and are candidates with AutD gene(s). Currently, we are investigating CpG island SNPs in this region for association with AutD. This island rescue system will allow us to investigate the methylation status and alterations of genes within the AutD region in tissues.

Program Nr: 2353 from the 2001 ASHG Annual Meeting

**Genetic analysis of attention network efficiency.** *J.A. Fossella, J. Fan, Y. Wu, T. Sommer, D. Pfaff, M.I. Posner.*  
Sackler Institute for Developmental Psychobiology, Department of Psychiatry, Weill Medical College, Cornell University, New York, NY.

Attention can be viewed in terms of neural networks that carry out specific functions. Each of these networks appears to be modulated by somewhat different neuromodulatory system. Studies of alert monkey suggest that the effectiveness of alerting produced by a warning signal can be eliminated by drugs that block noradrenaline. The executive network involves the anterior cingulate, which is modulated by dopamine from the ventral tegmental area. Our genetic analysis seeks to determine the contributions of noradrenergic and dopaminergic genetic variation to the efficiency of alerting and executive networks, respectively. As a first step, the heritability of both networks was determined. The Attention Network Task (ANT) was administered to 26 MZ twins and 26 age matched and same sex DZ twins. For the executive network (incongruent minus congruent RTs) the MZ twins showed a correlation of (0.73) and the DZ twins of (0.28). For this network the heritability was estimated ( $h^2 = 0.90$ ). For alerting, both MZ twins (.46) and DZ twins (.37) showed high correlations producing a much lower estimate of heritability ( $h^2 = 0.18$ ). ANT data have also been collected for a population of normal subjects ( $N = 200$ ). PCR-RFLP analysis of buccal swab DNA was used to detect variation in genes involved in dopaminergic and noradrenergic signaling. Results relating polymorphisms in these genes to the appropriate networks using ANT RTs will be presented.

**Chromosome 21 Promoter Activity and Polymorphism Effects.** *S.L. Coleman, C.A. Guy, B. Hoogendoorn, S.K. Smith, M.C. O'Donovan, P.R. Buckland.* Psychological Medicine, UWCM, Cardiff, Wales.

Chromosome 21 was the first chromosome to be completely sequenced and for this reason was chosen to identify putative promoters using the NCBI database. A total of 70 putative promoters were identified of which 50 were optimised for PCR. In order to confirm that the sequences identified were promoter regions all 50 PCR products were pooled in equal concentrations, ligated into a pGL3 T-vector and transformed. Sequencing identified 20 clones with the correct insert orientation. The pGL3 vector permits a simple luciferase assay to be carried out on cells that are transfected with pGL3 plasmids containing the test promoter, where the luciferase activity can be read in a luminometer. In comparison to the negative control of basic-pGL3, 90% were proven to have promoter activity in the three cell lines transfected (HEK 293t, JEG-3 and TE 671) and 20% showed higher promoter activity than the control pGL3-SV40. Due to the successful identification of promoter regions in the initial findings the original 50 putative promoters were screened with DHPLC for common polymorphisms using a screening set of 16 individuals. Of the 50 putative promoters 29 were identified as heterozygous. Each allele of the polymorphism was cloned into pGL3 and again transfected into the 3 cell lines. The luciferase activity for each allele was compared with its pair to see the effect of the polymorphism on promoter activity, with significant differences identified.



**Identification of a Molecular Variant at the Promoter Region of Human Reelin Gene and Association Study with Schizophrenia.** *C.H. Chen<sup>1,2</sup>, M.L. Chen<sup>1</sup>, S.Y. Chen<sup>3</sup>, C.H. Huang<sup>2</sup>*. 1) Dept Psychiatry, Tzu-Chi General Hosp, Hualien City, Taiwan; 2) Institute of Human Genetics, Tzu Chi University, Hualien, Taiwan; 3) Department of Life Sciences, Tzu Chi University, Hualien, Taiwan.

Reelin protein is essential for embryonic brain development as guiding the normal neuron migration and correcting cortical lamination. Recent studies indicated that reduction of reelin expression in the brain might be a vulnerability factor in schizophrenia. We hypothesized that functional polymorphism at the promoter region of the reelin gene might confer genetic susceptibility for schizophrenia. We searched for molecular variants at the promoter region of human reelin gene using single strand conformation polymorphism analysis, PCR direct sequencing and restriction fragment length polymorphism analysis in a cohort of Chinese schizophrenic patients from Taiwan. A G to C single nucleotide polymorphism at nucleotide position 888 upstream from translation initiation site was identified. This polymorphism was found to alter the Sp1 and/or hsp70.2 transcription factor binding sites by computer analysis. Further case control association study, however, did not reveal differences of genotype and allele frequency distributions between schizophrenic patients and comparison subjects. Our data suggest that this polymorphism at the promoter region of the reelin gene is not associated with schizophrenia, and there might be other factors that account for the decreased reelin expression in the brain of schizophrenic patients.

**Defective Inhibition of b-Catenin Signaling in Variants of SOX1 from Spina Bifida Patients.** *J.S. Nye<sup>1,4</sup>, L. Kan<sup>1</sup>, J. Luo<sup>1</sup>, V. Reddy<sup>1</sup>, S.G. Guy<sup>1</sup>, Z. Gu<sup>1</sup>, M. Matsumata<sup>2</sup>, Y. Kamachi<sup>2</sup>, J. Charrow<sup>1,4</sup>, J.F. Sarwark<sup>1,4</sup>, H. Kondoh<sup>2</sup>, M.C. Speer<sup>3</sup> and NTD Collaborative Group.* 1) Northwestern Univ Medical Sch, Chicago, IL; 2) Institute of Molecular and Cellular Biology, Osaka University, Osaka 565, Japan; 3) Center for Human Genetics, Duke University Medical School, Durham NC 27710, USA; 4) Childrens Memorial Hospital, Chicago IL.

Multiple genes are likely to contribute to the risk of neural tube defects (NTDs), but few genetic risk factors have been identified. Since deletions of a critical region of chromosome 13q33-34 are associated with NTDs, this region may contain one or more genes that are defective in patients with normal karyotypes. SOX1 is a Sry-class transcription factor encoded on 13q34 that is expressed in the neural plate, promotes neural development in model systems, and contains short imperfect repeats encoding one polyglycine and four polyalanines. Here we identify in-frame insertions and deletions in spina bifida patients and controls in these repetitive regions, revealing that the SOX1 protein is highly variable. Unique variants and genotypic combinations were identified in patients, but these were too rare to test for their contribution to risk. However, two of the alleles found in patients were defective in their ability to suppress b-catenin signal transduction, despite their ability to bind b-catenin and their ability to activate a target of group B SOX proteins, the d-crystalline promoter. These two variant SOX1 proteins functioned as dominant negatives when combined with wildtype proteins. Other variants showed a spectrum of activity in suppressing b-catenin signaling. These data suggest that defective modulation of b-catenin signaling may exist in individuals with spina bifida carrying certain SOX1 variants. Since numerous studies have shown that b-catenin signaling and the Wnt/wg pathway are critical for early neural development, these observations imply that variant forms of SOX1 may contribute to neural tube defects. Supported by the NICHD and NINDS.

**SNP analysis of candidate genes for isolated cleft lip and palate.** *R. Ingersoll<sup>1</sup>, S. Rowe<sup>1</sup>, M. Campbell<sup>1</sup>, L. Kasch<sup>1</sup>, I. McIntosh<sup>1</sup>, T. Beaty<sup>2</sup>, A.F. Scott<sup>1</sup>.* 1) Institute of Genetic Medicine, Johns Hopkins University School of Medicine; 2) Department of Epidemiology, School of Public Health, Johns Hopkins University.

We are investigating the genetics of non-syndromic cleft lip and palate by identifying single nucleotide polymorphisms (SNPs) in candidate genes identified by linkage analysis, animal models or presumed function. SNPs are identified in coding region, 3' UTR and promoter sequences by PCR amplification and direct sequencing of DNAs from unrelated parents of 15 probands. We are taking advantage of the increasingly complete human genome sequence and NCBI resources such as OMIM, LocusLink and Map Viewer to identify genes and genomic sequence. Sequences are aligned manually and visually inspected for differences. As of June 2001, PCR primers have been designed for 408 exons from 44 genes. Three genes have been completely sequenced (45 amplicons) and 20 additional exons have been completed from other genes. A total of 92 SNPs have been identified of which 18 occur in exons and eight of which result in amino acid changes. Allele frequencies will be determined for each SNP in a collection of trios obtained through a proband with a non-syndromic oral cleft (cleft lip with or without cleft palate and cleft palate only). Previous studies of these case-parent trios have shown evidence of linkage in the presence of disequilibrium for several candidate genes, in particular MSX1 and TGFB3. Both of these genes have been screened and a number of novel SNPs were identified.

**An association study of SNAP-25 alleles shows evidence for biased paternal inheritance in Attention Deficit Hyperactivity Disorder.** V. Kustanovich<sup>1</sup>, B. Merriman<sup>1</sup>, L. Crawford<sup>1</sup>, S. Smalley<sup>2</sup>, S. Nelson<sup>2</sup>. 1) Dept Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) UCLA Center for Neurobehavioral Genetics, UCLA, Los Angeles, CA.

**Research Goals:** Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common childhood neuropsychiatric disorders with an estimation of 3%-10% of school age children affected worldwide. Although the biological basis of this disorder is unknown, there is persuasive evidence that susceptibility to ADHD is in part genetically inherited. A recently implicated gene is the synaptic vesicle docking fusion protein, synaptic-associated protein of 25 kDa (SNAP-25). SNAP-25 has been shown to be causal in the hyperactive behavior of the mouse mutant *coloboma* (Cm/+). We have sought to define a possible association between this gene and ADHD.

**Methods:** All child and adolescent probands were assessed by direct interview using the K-SADS-PL. All adult probands and parents were assessed by direct interview with the SADS-LA-IV. Parental reports of child symptoms were obtained and, for children older than 8 years old, self-reports of symptoms were also obtained. In all cases, the structured interviews were supplemented with teacher and parent rating scales. Experienced clinical psychologists, trained on instruments of ADHD diagnosis, carried out all the interviews. We have implemented a fluorescence polarization template directed incorporation assay of single nucleotide polymorphisms (SNPs) in order to genotype over 550 individuals. Genotypes were gathered by AlleleCaller. Mendelian inheritance was verified by CheckMendel and TDT analysis was performed by TDT-SNPster.

**Results:** Here we report the confirmation of a genetic association between ADHD and two polymorphisms of SNAP-25 (T1065G and T1069C). We have tested and confirmed this association within multiplex families within our sample by the transmission disequilibrium test ( $c^2 = 4$ ,  $P = 0.045$ ). Further, we find convincing evidence of a paternal transmission bias of the SNAP-25 haplotype to affected children ( $c^2 = 9$ ,  $P = 0.003$ ).

**Codon 12 polymorphism of the peroxisome proliferator-activated receptor  $\gamma$  gene is not associated with body mass index or breast cancer.** A. Memisoglu<sup>1</sup>, S.E. Hankinson<sup>1,2</sup>, J.E. Manson<sup>2,3</sup>, G.A. Colditz<sup>1,2</sup>, D.J. Hunter<sup>1,2</sup> and Nurses Health Study. 1) Epidemiology, Harvard School of Public Health, Boston, MA; 2) Channing Laboratory, Dept. of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA; 3) Preventive Medicine, Dept. of Medicine, Harvard Medical School, Boston, MA.

Estrogen-induced cell proliferation is thought to underlie breast carcinogenesis. In addition to its role in the transcriptional regulation during adipocyte differentiation, studies indicate that the peroxisome proliferator-activated receptor $\gamma$  (PPAR $\gamma$ ) transcription factor is involved in transcriptional regulation of aromatase, a key enzyme in estrogen biosynthesis. Specific agonists for PPAR $\gamma$  induce differentiation and suppress markers of malignancy in breast cancer cells suggesting that PPAR $\gamma$  may be involved in breast carcinogenesis. The proline to alanine polymorphism in codon 12 (Pro12Ala) of PPAR $\gamma$  affects PPAR $\gamma$ 's DNA binding and transactivation ability. Here we determined the association of the Pro12Ala PPAR $\gamma$  polymorphism with breast cancer in a case-control study nested within the Nurses Health Study. 725 incident cases of breast cancer and 953 matching controls were included and no association was observed between PPAR $\gamma$  Pro12Ala polymorphism and breast cancer (odds ratio of 1.08 (0.85-1.38) for variant allele carriers compared to non-carriers). Further, serum levels of estrone sulfate, estrone, estradiol, testosterone and androstenedione were similar among PPAR $\gamma$  genotypes. In contrast to some reports, BMI of individuals in this study did not vary with PPAR $\gamma$  genotype. However, an association between PPAR $\gamma$  genotype and age at menarche was observed. Mean age of menarche for homozygous individuals bearing only the variant alanine allele was 13.32 years compared to 12.49 and 12.52 years for heterozygotes and homozygotes bearing only the common proline allele, respectively ( $p=0.005$ ). Thus, although an association between the Pro12Ala PPAR $\gamma$  polymorphism and plasma estrogens or breast cancer risk was not observed, this genetic variant may alter estrogen-dependent developmental programs such as the timing of menarche.

**The (TG)<sub>n</sub> promoter polymorphism in the NRAMP1 gene and susceptibility to sarcoidosis.** *T.A. Wischmeijer<sup>1</sup>, C. Bombieri<sup>1</sup>, M. Luisetti<sup>2</sup>, P.F. Pignatti<sup>1</sup>.* 1) Section of Biology & Genetics, DMIBG, University of Verona, Verona, Italy; 2) Inst. Respiratory Diseases, IRCCS, S.Matteo, University of Pavia, Pavia, Italy.

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology, that may represent an immunologic response to an exogenous agent in a genetically susceptible individual. The human natural resistance-associated macrophage protein 1 (NRAMP1) gene encodes a pH-dependent divalent cation transporter involved in macrophage function. A functional repeat polymorphism in the promoter of NRAMP1 has been described. In different populations, allele 2 occurs at an allele frequency of about 0.20-0.25 and has been correlated to infectious disease susceptibility. Allele 3 occurs at an allele frequency of about 0.75-0.80, and has been associated with susceptibility to autoimmune disease. The other described alleles are rare (allele frequencies less than 0.001). In a report on a case-control study in African American sarcoidosis patients, Maliarik et al. (*Am J Respir Cell Mol Biol* 2000;22:672-675) suggested that allele 2 may decrease susceptibility to sarcoidosis. We performed a case-control association study in 51 Italian sarcoidosis patients and 80 Italian control subjects, to test whether the (TG)<sub>n</sub> promoter polymorphism in NRAMP1 is associated with susceptibility to sarcoidosis. Individuals with genotype 3,3 were more common in sarcoidosis: 29/51 (57%) vs. 33/80 (41%) in controls. The difference is not statistically significant. Of 102 alleles of sarcoidosis patients, we found 22/102 (22%) alleles 2 and 77/102 (75%) alleles 3. There were 3/102 (3 %) other alleles. In 160 control alleles 50/160 (31%) could be identified as allele 2 and 107/160 (67%) as allele 3. We found 3/160 (2%) other alleles in the control group. In accordance with Maliarik et al., we found a decreased frequency of allele 2 in sarcoidosis patients compared to controls. However, we can't support the hypothesis of a protective effect of allele 2, since the results in our population are not statistically significant.

**Association study of polymorphisms in the glutamate receptor genes, *GRM2* and *GRIK1* with Japanese schizophrenia.** H. Shibata<sup>1</sup>, A. Joo<sup>1</sup>, Y. Fujii<sup>1</sup>, A. Tani<sup>1</sup>, C. Makino<sup>1</sup>, N. Hirata<sup>1</sup>, R. Kikuta<sup>1</sup>, H. Ninomiya<sup>2</sup>, H. Kawasaki<sup>2</sup>, N. Tashiro<sup>2</sup>, Y. Fukumaki<sup>1</sup>. 1) Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, JAPAN; 2) Department of Neuropsychiatry, Graduate School of Medical Sciences, Kyushu University, Fukuoka, JAPAN.

Upon the glutamatergic dysfunction hypothesis for schizophrenia, glutamate receptor genes are strong candidates for susceptibility genes of the disorder. We screened polymorphisms in two glutamate receptor genes: the metabotropic glutamate receptor type 2 gene, *GRM2* and the kainate receptor type 1 gene, *GRIK1*, and examined putative association of the polymorphisms with schizophrenia. We recruited both schizophrenics (n = 213) and healthy controls (n = 220) from Kyushu area in Japan. All specimens are unrelated and ethnically Japanese. We identified 13 SNPs (ten missense, one synonymous, two in the noncoding exon) in the entire exons and four polymorphisms (one SNP and three indels) in the 5' upstream region of *GRM2* by PCR-SSCP and direct sequencing. We genotyped the entire samples of both schizophrenics and controls for all the 17 polymorphic sites. The observed frequencies of the polymorphisms were low (< 2 %) both in schizophrenics and controls. There is no significant differences in allele frequencies of all tested polymorphisms in *GRM2*. We also identified six SNPs (three missense, three synonymous) in the entire exons of *GRIK1* by the same methods. Since only three SNPs (one missense, two synonymous) among the six were commonly observed in the polymorphism scanning process (> 7 %), the extensive genotyping for the three major SNPs in *GRIK1* was performed against the entire samples of schizophrenics and controls. From the observed allele frequencies, we estimated haplotype frequencies of the three SNPs using the EH program. There is no significant association of schizophrenia with the SNPs and their haplotypes in *GRIK1* locus. Therefore, we conclude that neither *GRM2* nor *GRIK1* plays a major role in schizophrenia pathogenesis in the Japanese population.

**Major structural polymorphisms in the nicotinic acetylcholine receptor  $\alpha$ -7 Subunit (CHRNA7) partial duplication on chromosome 15q13-q14.** *B.P. Riley<sup>1</sup>, M. Williamson<sup>2</sup>, D. Collier<sup>3</sup>, H. Wilkie<sup>3</sup>, A. Makoff<sup>3</sup>.* 1) Dept. of Psychiatry, Virginia Commonwealth Univ., Richmond, VA; 2) Department of Paediatrics, University College London Medical School, London, UK; 3) Division of Psychological Medicine, Institute of Psychiatry, De Crespigny Pk., London, UK.

We have reported evidence of linkage and haplotype transmission disequilibrium between schizophrenia and markers in the region of CHRNA7 and its nearby partial duplication. The 95-99% identity between gene and duplication, and between the multiple copies of novel exons associated with the duplication, necessitated the identification of unique positions in this complex region of chromosome 15q13-q14. We identified five distinct sequences, four of which map to 15q13-q14, from 5'-RAGE clones: those representing the gene and duplication, two distinct versions of the novel exons, and an unknown kinase gene mapped to chromosome 3 from which most but not all of the novel exons originate. The physical map assembled from these clones reveals two kinds of variation involving major structural differences. First, the partial duplication is polymorphic. Second, where present, it can exist in either orientation. Finally, a number of previously identified sequence variants, including a 2 bp deletion in exon 6, exist in the duplication. We are currently developing assays to determine the copy number and orientation of the partial duplication using real time PCR and multicolor FISH in order to obtain detailed genotypes and haplotypes of variants in the region in our family sample.



**Analysis of the Leptin receptor SNP, GLN223ARG, in anorexia nervosa.** *D.W. Meechan<sup>1</sup>, N.D. Quinton<sup>2</sup>, L.F. Pieri<sup>3</sup>, K.M.O. Brown<sup>4</sup>, H.E. Eastwood<sup>4</sup>, A.I.F. Blakemore<sup>5</sup>*. 1) Biomedical Research Centre, Sheffield Hallam University, Sheffield, S1 1WB UK; 2) School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT, UK; 3) Yorkshire Centre Eating Disorders, Seacroft Hospital, Leeds, UK; 4) Molecular Medicine Unit, University of Leeds, St James Hospital, Leeds, UK; 5) Imperial College of Science, Technology and Medicine, Kennedy Galton Centre, Level 8V, Northwick Park Hospital, Watford Road, Harrow, HA1 3UJ, UK.

Anorexia nervosa is a complex disorder of unknown aetiology, characterised by abnormal eating and purging behaviours with the express aim of reducing body weight. Leptin, a cytokine expressed and secreted by the adipose tissue, is involved in the regulation of body weight. Recent reports have indicated that leptin levels are reduced in underweight anorexic patients and these levels correlate with decreased body mass index (BMI). During recovery, leptin levels progressively increase to reach higher than expected levels for the body weight.

We, and others, have shown an association between the leptin receptor SNP, GLN223ARG, and fat mass, body mass index (BMI) and leptin levels in Caucasian post-menopausal women. The aim of this study was to determine whether this association could also be found in young female, Caucasian control subjects and whether this SNP is associated with anorexia nervosa. 175 subjects with anorexia nervosa (divided into bingeing/purging and restricting anorexics) were recruited from the Yorkshire Centre for Eating Disorders at Seacroft Hospital. 145 controls were recruited from the University of Leeds. In both cohorts, genotype and allele frequencies for GLN223ARG, did not differ significantly from published frequencies. There was no significant difference in genotype frequency between the control and the anorexic cohorts or between restricting and bingeing subjects with anorexia and there was no correlation with BMI in patients or controls. This may reinforce existing evidence that this SNP is associated with adiposity in middle-aged, but not young subjects.

**Association study of SNPs in 26 candidate genes involved in blood pressure variation in an isolated population.** *L. Jin*<sup>1,2,3</sup>, *Z. Jiang*<sup>2,3</sup>, *J. Shi*<sup>3</sup>, *H. Chen*<sup>2</sup>, *J.M. Akey*<sup>1</sup>, *W. Huang*<sup>3</sup>. 1) Environmental Health, University of Cincinnati, Cincinnati, OH; 2) Institute of Genetics, Fudan University; 3) National Human Genome Center at Shanghai.

Twenty-six candidate genes implicated in blood pressure (BP) regulation were selected, fifteen from candidate regions based on whole genome scanning and linkage disequilibrium analyses and eleven based on their biological functions. We identified 286 SNPs in the promoter regions, exons, and intron-exon junctions of these genes (124kb in length) by sequencing 80 individuals with blood pressure chosen from the two tails of the blood pressure distribution in an isolated Chinese population. Approximately 21 SNPs detected in 9 genes showed significant allele frequency differences between the high and low BP groups. Extended genotyping in larger samples showed that the association between four genes and BP remained. The genetic diversity of this isolated population is generally smaller compared to African and European populations. The majority of the SNPs found in this study are absent in the African and European populations challenging the rationale of the recent SNP discovery effort. Further analyses showed that no population expansion or population stratification was present in this population, while evidence for natural selection is also absent in all the genes studied except one.

**Functional analysis of promoter variants in the  $\alpha 7$  nicotinic receptor gene in normal and schizophrenic individuals.** *J.M. Gault<sup>1</sup>, J. Logel<sup>2</sup>, C. Drebing<sup>1</sup>, J. Hopkins<sup>2</sup>, M. Short<sup>2</sup>, R. Vianzon<sup>1</sup>, D. Venn<sup>1</sup>, K. Walton<sup>1</sup>, R. Berger<sup>1</sup>, B. Sullivan<sup>1</sup>, M. Maslak<sup>2</sup>, M. Robinson<sup>1</sup>, R. Freedman<sup>1,2</sup>, S. Leonard<sup>1,2,3</sup>.* 1) Dept Psychiatry, Univ Colorado Health Sci Ctr, Denver, CO; 2) VA Medical Center, Denver, CO; 3) Dept Pharmacology, Univ Colorado Health Sci Ctr, Denver, CO.

The  $\alpha 7$  neuronal nicotinic receptor subunit (CHRNA7) has an important role in the P50 auditory gating deficit found in many schizophrenics as shown by pharmacological investigation in humans and rodents. Samples from 167 controls and 192 schizophrenics of Caucasian (64%), African American (32%) and Hispanic (4%) descent were analyzed for proximal promoter mutations. Sequence and single strand conformational polymorphism (SSCP) analysis identified 11 unique single bp variants, 11 different combinations of double bp variants and 2 individuals with multiple base insertions in the 231bp promoter region upstream of the ATG translation start site. Functional analysis of 8 polymorphisms, using a luciferase reporter gene assay in transfected SHSY-5Y neuroblastoma cells, shows a statistically significant drop in the transcriptional activity of 8 to 38% for 6 of the 8 variants examined ( 86C@T, 92G@A, 143G@A, 178delG, -194G@C, and 241A@G). These six variants were more prevalent in schizophrenics than controls (controls 15% vs. schizophrenics 26%,  $p=0.0137$ ). The most frequent variant, 86 C@T, decreases promoter activity by 18% ( $p<0.0001$ ) and is associated with schizophrenia ( $p=0.0288$ ) in a sample of combined ethnic groups. The controls with no variants have a significantly lower mean P50 ratio than controls in whom a promoter variant was found ( $p=0.0001$ ). These results suggest that some  $\alpha 7$  promoter variants may be functional and are related to the auditory gating phenotype.

**Coronary atherosclerosis: correlation of the FGF2 gene-expression to genetic variants in the 5UTR.** *P. Greiser<sup>1</sup>, U. Schagdarsurengin<sup>1</sup>, S. Schulz<sup>1</sup>, T. Süß<sup>2</sup>, A. Kabisch<sup>3</sup>, D. Rehfeld<sup>2</sup>, U. Müller-Werdan<sup>2</sup>, B.M. Taute<sup>2</sup>, K. Werdan<sup>2</sup>, I. Hansmann<sup>1</sup>, C. Gläser<sup>1</sup>.* 1) Inst Human Genetics, Univ Halle, Halle, Germany; 2) Dep Intern Med, Univ Halle, Halle, Germany; 3) Inst Transf Med/Bloodbank, Univ Halle, Halle, Germany.

The potent cytokine FGF2 is involved in the proliferative response to vascular injury of many cell types like monocytes/macrophages, fibroblasts, vascular smooth muscle and endothelial cells and is suggested as an important candidate gene for CAD. The C223T polymorphism of its exon1, located in the 5' untranslated region (5UTR) in the functionally important internal ribosome entry site, modifies the calculated mRNA folding-structure and is regarded to influence the transcription and translation. We included 152 probands (118 males, 49.92y, SD 9.02) with angiographically proven coronary state (91 CAD, 61 controls with unaffected coronary vessels), determined their C223T genotypes (SSCP) and detected their in vivo FGF2 mRNA-expression in monocytes (competitive RT-PCR). **Results:** We found significantly higher expression levels in the CAD patients compared to the coronary controls (1.19ag/U vs. 0.86ag/U,  $p < 0.03$ ). Analyzing all probands the highest expression was detected in the frequent wildtype carrier CC (frequency 0.82,  $n = 125$ ) with 1.13ag/U whereas the T-allele carriers showed lower levels (CT,  $n = 25$ , genotype frequency 0.17, expression 0.77ag/U) (TT,  $n = 2$ , genotype frequency 0.01, expression 0.25ag/U) ( $p < 0.015$ ). The same tendency was found in both subgroups (CAD: CC/CT/TT 1.28/0.77/0.30ag/U,  $p < 0.02$ ; controls CC/CT 0.89/0.77ag/U, n.s.).

**Conclusion:** In general the frequent wildtype carriers (CC) are characterized by a high level of the FGF2 gene-expression in monocytes, a crucial cell system in the atherosclerosis development. CAD is associated with an additional 43% up regulation in CC carriers. T-allele carriers seem to be unable to realize such a regulation: independent of the coronary findings both CAD patients and controls had comparable and lower FGF2 mRNA-levels, and the lowest showed homozygous TT carriers. Our results suggest that the cellular FGF2 expression could be a sufficient risk marker dependent on C223T gene-constellation.

**Heterozygosity for a surfactant protein C mutation causes familial usual interstitial pneumonitis in adults and cellular interstitial pneumonitis in children.** *A. Thomas<sup>1,5</sup>, K. Lane<sup>1,5</sup>, J. Loyd<sup>1,5</sup>, M. Prince<sup>2,5</sup>, C. Markin<sup>1,5</sup>, J. Johnson<sup>3,5</sup>, J. Haines<sup>4,5</sup>, M. Stahlman<sup>2,5</sup>, J. Phillips III<sup>2,5</sup>.* 1) Department of Medicine; 2) Department of Pediatrics; 3) Department of Pathology; 4) Department of Molecular Physiology and Biophysics; 5) Vanderbilt University School of Medicine, Nashville, TN.

Idiopathic pulmonary fibrosis (IPF) is a fibrotic, destructive lung disease of unknown cause. A familial occurrence of the disease (FIPF) has been reported in up to 25% of cases. A mutation in the gene (SFTPC) encoding surfactant protein C (SP-C) has recently been described in an infant and mother with interstitial lung disease. We hypothesized that FIPF is caused by a mutation in SFTPC. In one large FIPF family, we performed a mutational and expression analysis of SP-C by genetic linkage, dideoxy-fingerprinting (ddF), sequencing, and immunohistochemistry. This family contains 14 affected members with interstitial lung disease, including adults with usual interstitial pneumonitis (UIP) and children with cellular interstitial pneumonitis (CIP). All affected family members shared the same polymorphic marker located near SFTPC. Linkage analysis generated a lod score of 4.33. DNA from all affected individuals studied shared an abnormal ddF pattern. Sequencing of this region revealed an Exon 5+128 T to A transversion that was present in the DNA of all 6 affected family members studied and 2 obligate carriers by restriction enzyme analysis, but in none of 88 control chromosomes. This mutation substitutes a glutamine for a highly conserved leucine residue that is predicted to hinder post-translational clipping of SP-C precursor (proSP-C), which would cause its retention in the endoplasmic reticulum. Staining for proSP-C by immunohistochemistry in lung tissue from an affected individual revealed dysplastic, proliferating type II alveolar cells with abundant intracellular proSP-C. Our findings show that a SFTPC mutation segregates with FIPF. The presence of UIP and CIP in affected relatives sharing this mutation indicates that these diseases may represent pleiotropic manifestations of the same central pathogenesis.

**Delineation of the Mechanism of Glycine N-methyltransferase Gene Control in Liver Cancer.** *Y.A. Chen<sup>1</sup>, C.-M. Lee<sup>1</sup>, Y.-P. Shih<sup>1</sup>, T.-L. Tseng<sup>2</sup>, K.H. Buetow<sup>2</sup>.* 1) Inst Public Health, Natl Yang-Ming Univ, Taipei, Taiwan; 2) Laboratory of Population Genetics, Division of Cancer Epidemiology and Genetic, NCI, Bethesda, MD. 20892, USA.

Glycine N-methyltransferase (GNMT) is not only an enzyme regulating the concentration of the methyl-group donor, S-adenosylmethionine, but also is the 4S polycyclic aromatic hydrocarbon-binding protein participating in the detoxification pathway of cells. Previously, we reported that the expression of GNMT was diminished in human hepatocellular carcinoma (HCC) cell lines and tissues. Genotypic study and liver cancer susceptibility analyses showed that the average of odds ratio for people who possess risk genotypes of GNMT to develop HCC was 1.37~3.97 (see Dr. Tzu-Ling Tseng's abstract in this meeting). In addition, all five HCC cell lines including HA22T, Hep 3B, HuH-7, Sk-Hep1 and PLC/PRF-5 had homozygous genotypes on the polymorphism markers in the promoter region while two hepatoblastoma cell lines (HuH-6 and HepG2) were heterozygous on GNMT gene. Methylation-sensitive restriction endonuclease digestion and PCR amplification demonstrated that among 7 cell lines mentioned above, the upstream control region of GNMT gene was methylated in 4 cell lines (HA22T, Sk-Hep1, PLC/PRF-5 and HuH-6). The GNMT upstream promoter region was subcloned to a vector-pGL3-Basic containing the luciferase gene. Plasmid DNA from the resultant plasmid-pGNMT1.8k was used to transfect different cell lines and showed that the luciferase activity ratios of pGNMT1.8k vs. pGL3-Basic were 18.7 for HepG2 and 2.0 for a subclone of HepG2 that expresses HBV viral antigens (MS-G2). The interaction between GNMT gene control and HBV was further investigated by cotransfection experiment. In comparison with the cells cotransfected with vector DNA, the luciferase activity decreased 67% in the cells cotransfected with pGNMT1.8k and a plasmid containing HBV full genome-pHBV1.3-Bcl. Therefore, loss of heterozygosity, DNA methylation and HBV infection may contribute to the down-regulation of GNMT gene expression during the tumorigenesis of liver.

**Characterization of the Aryl Hydrocarbon Receptor Repressor Gene and Association of Its Pro185Ala**

**Polymorphism with Micropenis.** *H. Fujita*<sup>1</sup>, *R. Kosaki*<sup>2</sup>, *H. Yoshihashi*<sup>1</sup>, *T. Ogata*<sup>1</sup>, *N. Matsuo*<sup>1</sup>, *K. Kosaki*<sup>1</sup>. 1) Dept of Pediatrics, Keio University School of Medicine, Tokyo, Japan, 160-8582; 2) Dept of Genetics, Saitama Children's Medical Center, 2100 Magome, Iwatsuki, Japan, 339-8551.

In utero exposure to dioxins affects male external genital development in rodents. The effects of dioxins are mediated via the aryl hydrocarbon receptor (AHR) and its binding protein, aryl hydrocarbon receptor nuclear translocator (ARNT). In mice, aryl hydrocarbon receptor repressor (AHRR), which binds to ARNT in competition with AHR, plays a critical negative regulatory role in mice AHR signalling. We attempted to characterize the human AHRR gene and investigate the relationship between AHRR polymorphisms and the incidence of micropenis, a phenotype of demasculinization. We identified and characterized the human homolog of mouse AHRR, taking advantage of the publicly available draft version of the human genome sequence. The deduced sequence for human AHRR (715 residues) and the mouse AHRR protein exhibited 81 % sequence homology to each other. Radiation hybrid mapping revealed that AHRR was located on 5pter. This location was syntenic to chromosome 13 in mouse genome according to the mouse-human homology map. We then detected an AHRR protein polymorphism by the direct sequencing of pooled human genomic DNA. Pro185Ala polymorphism was identified between the PAS-A region and the highly conserved arginine/cysteine-rich RCFRCRL/VRC region, which may affect the inhibitory action of AHRR on AHR signalling by altering the heterodimerization of AHRR and ARNT or by altering DNA binding. We evaluated the association between the polymorphism and the presence or absence of micropenis ( $< 2.5SD$ ) in patients with micropenis and control subjects. Forty-six percent (27/59) of patients with micropenis and 27% (22/80) of the controls were homozygous for 185Pro; this difference in frequencies was significant ( $P = 0.03$ ). This result indicates that the homozygosity for the 185Pro allele of AHRR may increase the susceptibility of a fetus to the demasculinizing effects of dioxin exposure in utero, presumably through the diminished inhibition of AHR-mediated signalling.

**Valproic acid exposure alters gene expression in rat embryos: mechanism of teratogenicity and relationship to autism spectrum disorders.** C.J. Stodgell, S. Gnall, P. Rodier. Dept OB/GYN, Univ Rochester School of Medicine, Rochester, NY.

Valproic acid (VPA) exposure during pregnancy is associated with physical malformations and behavioral deficits, developmental delays and autism. Some of these terata can be reproduced in rats by in utero exposure to VPA. *Hoxa1* knockout mice display brainstem and craniofacial deficits similar to those seen in VPA-exposed rats and in cases of autism. In humans, *HOXA1* has been linked to increased susceptibility to autism and autistic behaviors. We postulated that interference with expression of *HOXA1* and other genes might be a mechanism of VPAs teratogenicity. To test this, we exposed rat embryos to a teratogenic dose of VPA or saline (control) during the period of *Hoxa1* expression (i.e., day 10.5-13.5 of gestation). At all of these times, VPA significantly increased the expression of *Hoxa1* relative to expression seen in saline-treated controls. VPA also induced abnormal expression of the gene prior to day 10.5 and as late as day 14, both times when *Hoxa1* is not expressed. By day 15, *Hoxa1* expression had ceased in controls, and could not be induced by VPA. In day 12 embryos, we compared the effects of exposure to a teratogenic analog of VPA (4-yn) or a nonteratogenic form (IE-VPA). 4-yn significantly ( $p < 0.005$ ) increased *Hoxa1* expression at 2, 4, 6 and 24 hours post-treatment compared to age-matched, saline-treated controls. IE-VPA did not alter expression of *Hoxa1* at any time point. DNA microarrays (Affymetrix RAT34A) were also used to examine the effect that VPA, 4-yn and IE-VPA had on expression of genes in embryos treated on day 12. Expression of several transcription factors, cell cycle and signal transduction genes were affected by VPA and 4-yn, and were not affected by IE-VPA exposure. These data suggest that VPA's effect on gene expression is a mechanism of VPA's teratogenicity, and they offer one explanation for why VPA exposure increases the risk of autism.



**Single-genome analysis of the expanded GAA triplet-repeat sequence indicates a very high mutation load *in vivo* and a distinct contraction bias.** S. Bhatti<sup>1</sup>, R. Clark<sup>1</sup>, T. Ashizawa<sup>2</sup>, S.I. Bidichandani<sup>1</sup>. 1) Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Neurology, Baylor College of Medicine, Houston, TX.

Friedreich ataxia (FRDA) is caused by abnormal expansion of a GAA triplet-repeat (GAA-TR) sequence in the first intron of the frataxin gene. Normal and mutant chromosomes have 6-60 and 100-1700 repeats, respectively. The expanded GAA-TR is known to be unstable in somatic and germ cells. To enable a detailed characterization of somatic instability we used a small-pool PCR (SP-PCR) assay that detected alleles ranging from 7-1677 repeats with single-genome resolution. Over 1000 individual leukocyte genomes were analyzed from five heterozygous carriers of expanded alleles with 798-1088 GAA triplets (progenitor allele sizes were determined by genomic Southern analysis). We detected a striking degree of somatic variability *in vivo*, with mutation loads (the proportion of alleles that differed significantly from the progenitor allele) ranging between 0.89-0.97 for the various expansions. Extreme changes in repeat length were identified; contractions and expansions up to 24% and 154% of the original progenitor allele length were seen. There was a distinct bias towards contraction, with a significant correlation between the proportion of contracted alleles and the length of the progenitor allele ( $r^2 = 0.82$ ). The mutational spectrum revealed two types of changes: small mutations (<5% of allele length) with equal prevalence of contractions and expansions, and large mutations (>15% of allele length) among which contractions were 2-3 times more frequent. This bias towards contraction is in contrast with the expansion bias displayed by similar alleles of the CTG triplet-repeat in myotonic dystrophy. Additionally, SP-PCR analysis of serially passaged lymphoblastoid cells also showed increasing mutation loads upon serial passaging and a preponderance of large contractions. In addition to the mechanistic implications of the deletional bias, these findings suggest the possible role of cellular development and proliferative capacity in mediating somatic instability of the GAA triplet-repeat.

**Unexpectedly low prevalence of Friedreich ataxia in the Mexican population.** *S.I. Bidichandani<sup>1</sup>, R.M. Clark<sup>1</sup>, S. Bhatti<sup>1</sup>, E. Alonso<sup>2</sup>, P. Yescas<sup>2</sup>, A. Rasmussen<sup>2</sup>.* 1) Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) Neurogenetics, Instituto Nacional de Neurologia y Neurocirugia Manuel Velasco Suarez, Mexico City, Mexico.

Friedreich ataxia (FRDA) is an autosomal recessive disease characterized by neurodegeneration, cardiomyopathy and diabetes. Over 95% of FRDA patients are homozygous for an abnormal expansion of a polymorphic GAA triplet repeat sequence in the first intron of the frataxin gene. Normal and mutant chromosomes have 6-60 and 100-1700 triplet repeats, respectively. In the Indo-European and North African populations, FRDA is the most common inherited ataxia, accounting for at least 50% of all recessive and sporadic ataxias. Almost 1% of the chromosomes in these populations carry pathologic GAA triplet-repeat expansions (>100 triplets), resulting in the observed disease incidence of 1-2 / 50,000. The expanded alleles in turn originate from long normal (LN) alleles (>12 triplets) which account for 10-15% of all Indo-European and North African chromosomes. In contrast, East Asians (from Japan, China, and South East Asia) and Native Americans have a significantly lower frequency of LN alleles (<2%), and consequently have a very low incidence of FRDA. No such data exists for the Mexican population. We analyzed a cohort of 88 patients with recessive (n = 36) and sporadic (n = 52) ataxia from Mexico and found only 5 individuals (5.7%) who were homozygous for pathologic GAA triplet-repeat expansions, consistent with the diagnosis of FRDA. Three of them were sporadic, and 2 were recessive cases. This indicates that FRDA represents a minority of all Mexican recessive and sporadic ataxia patients, significantly lower than the frequency observed in the Indo-European and North African populations. The lower prevalence of FRDA among Mexicans could stem from a lower frequency of LN alleles. Of the 176 chromosomes analyzed, we observed only 10 LN alleles (5.7%), representing a significantly lower frequency of LN alleles. These data have important epidemiological implications concerning the incidence of FRDA in Mexico, and in the U.S. among individuals of Mexican origin.

**Molecular analysis of a fragile X family with two females homozygous for a premutation.** *F.B. Essop<sup>1</sup>, J. Greenberg<sup>2</sup>, D. Basel<sup>2</sup>, A. Krause<sup>1</sup>.* 1) Department of Human Genetics, School of Pathology, South African Institute for Medical Research and University of the Witwatersrand, Johannesburg, South Africa; 2) Department of Human Genetics, University of Cape Town Medical School, Observatory, South Africa.

Fragile X syndrome (FRAXA) is the most common inherited cause of mental retardation. The disorder affects 1 in 5000 males. Fragile X results from the inheritance of an unstable DNA repeat (a CGG triplet repeat in the 5'-untranslated region of the FMR-1 gene) capable of expanding with successive generations. Normal individuals have 5-50 repeats, a premutation carrier 50-200 repeats and affected individuals have more than 200 CGG repeats. Repeat sizes from 40 to 60 repeats are considered to be a grey area/intermediate range. Alleles in this range may be transmitted stably or show instability. The clinical significance of carrying an allele in this intermediate size range is unclear, particularly for the smaller premutation sizes, but there may be an increased risk of expansion in the future generations.

We describe an interesting family in which two sisters are homozygous for a premutation (PM). Sister 1 (S1) has repeat sizes of 51 and 69 and sister 2 (S2) has repeat sizes of 51 and 57. The sisters inherited a premutation allele from each of their parents. The father carries an allele of 51 repeats and the mother carries alleles of sizes 29 and 57 repeats. S1 has a son affected with FRAXA. S2 has 4 children. In only one of them (a daughter) has her PM expanded to 67 repeats. Clinically the sisters are normal. To determine which allele of S2 underwent expansion, linked marker analysis using microsatellite markers DXS548 and FRAXAC2 was undertaken. The 57 allele in S2 was shown to have expanded.

Molecular detection for fragile X was undertaken by PCR and Southern blotting. This is the first South African report of females homozygous for a premutation and the fourth case in the literature.

**Comparison of the CAG-expansion content of the human and mouse genome using 2-D Repeat Expansion Detection (2D-RED).** *K.A. Benzow, M.D. Koob.* Inst Human Genetics, Univ Minnesota, Minneapolis, MN.

We have used two-dimensional Repeat Expansion Detection (2D-RED), a method that we have described previously, to analyze the approximate size and number of CAG expansions in the genomic DNA of both humans and mice. We consistently detect only five genomic fragments that generate RED signals of 30 CAG repeats (RED30) or greater in typical human genomic DNA samples. We have identified the source of these signals to be the androgen receptor gene (AR, RED30), the ataxin-1 gene (SCA1, RED30), the AF-9 gene (RED40), and the "background" CAG expansions MN1 and ERDA (RED50 and greater, present in almost half of the samples analyzed). When we used the same 2-D RED conditions to assay genomic DNA from standard laboratory mouse strains, however, we found that essentially all genomic size fractions generated RED30 or greater signals and that three size fractions generated RED90 or greater signals. We used our RAPID cloning method to identify the mouse genomic sequence that generated the largest RED signal and found that it was a stretch of 101 pure CAG repeats interrupted in 28 positions by GAG and in one position by GGA (i.e., 130 CAG repeats with 31 nucleotide changes). This polymorphic sequence is in the promoter region of the mouse cathepsin S (CatS) gene. RAPID cloning of sequences that generated some of the smaller signals identified both pure CAG repeat tracts and another CAG/GAG mixed tract similar to that in the CatS promoter. By reanalyzing the mouse genomic DNA using more stringent 2D-RED conditions, we were able to eliminate the signal from these CAG/GAG mixed sequences and detected only numerous RED30 size fractions and a single RED40 signal (mouse AF9). Our results indicate that genomic DNA from common lab strains of mouse 1) has more small, pure CAG expansions in the 20-30 repeat size range than does the human genome, 2) has many minimally interrupted repeats not seen in human genomic DNA, but 3) does not have large uninterrupted repeats found in many human genomic samples. These results are consistent the hypothesis that CAG expansions may be generated or repaired in a different manner in mice and in man.

**Resistance of an expanded polyglutamine repeat androgen receptor to 26S proteasome degradation results in amino-terminal fragment formation.** *I.F.M. Andriola<sup>1</sup>, C.K. Bailey<sup>1,2</sup>, D.E. Merry<sup>1</sup>*. 1) Dept. Biochem & Molec Pharm, Thomas Jefferson Univ, Philadelphia, PA; 2) Univ of Pennsylvania, Neuroscience Graduate Group, Philadelphia, PA.

Proteolysis is essential for execution of many cellular functions, including the removal of incorrectly folded or damaged proteins. For this purpose the 26S proteasome is the major proteolytic system of the nuclear and cytoplasmic compartments of eukaryotic cells, responsible for the degradation of many cellular proteins. Increasing evidence indicates that altered protein handling leads to impairment of proteasome activity, thus contributing to neuronal cell death in several neurodegenerative diseases. We have observed polyglutamine repeat length dependent proteolysis of the androgen receptor, coupled with cell death in a cell culture model of spinal and bulbar muscular atrophy (SBMA). Inhibition studies have revealed that the fragment production depends on the activity of the 26S proteasome. The proteasome inhibitors lactacystin and the highly specific epoxomicin can effectively block the production of amino-terminal fragments of AR. This "proteolysis" results in the production of a repeat containing amino-terminal AR fragment. Fractionation of expanded AR-expressing cells indicated that the repeat-containing amino-terminal fragment is exclusively nuclear. These results indicate that the presumptive proteolytic AR fragment represents the inefficient degradation of the expanded repeat AR, rather than the site-specific cleavage by another protease. The finding of a specific location within the expanded repeat AR protein resisting proteasome degradation indicates a specific structural site of proteasome failure. Furthermore, the overexpression of the molecular chaperons Hsp70 and Hsp40 eliminates amino-terminal fragment formation even while promoting AR degradation. This indicates that chaperone-mediated folding to a native AR conformation stably prevents the conformational change that blocks processive AR proteasome degradation. These results suggest that dysfunction of the ubiquitin-proteasome pathway may be involved in cell death induced by an expanded polyglutamine AR.

**Identification of a boundary between imprinted Prader-Willi syndrome genes and 3 adjacent non-imprinted genes.** *J.H. Chai*<sup>1</sup>, *J.M. Greally*<sup>2</sup>, *J. Dunai*<sup>2</sup>, *D.P. Locke*<sup>3</sup>, *T. Ohta*<sup>1</sup>, *E.E. Eichler*<sup>3</sup>, *R.D. Nicholls*<sup>1</sup>. 1) Univ. Pennsylvania, Philadelphia, PA; 2) Albert Einstein Coll. Med., New York, NY; 3) Case Western Reserve Univ., Cleveland, OH.

Prader-Willi syndrome (PWS) shows loss of function of paternally expressed genes in a 2 Mb imprinted domain of human chromosome 15q11-q13 or the homologous mouse 7C, with imprinting controlled in *cis* by an imprinting center (IC). Previous studies identified an imprinted, intronless gene cluster ~1 Mb from the IC and a flanking non-imprinted gene, *SRAI*, at an unknown distance. To characterize this region further, we searched for CpG islands near *SRAI* and identified 2 novel genes, *MNCb-2146* (*M2146*; 7 exons) and *MNCb-2146-LIKE* (*M2146L*; 5 exons). A (GCG)<sub>n</sub> coding polymorphism in *M2146L* exon 1 varies from n=6-10 (mean 7-8) in 30 individuals of European and Asian origin. While the functions are unknown, the human and mouse 329 amino acid (aa) M2146L or 360 aa M2146 polypeptides show 99% or 96% identity, respectively, with 43% identity for the paralogous pairs. Human *M2146L* expresses 2.2- and 7.5-kb mRNAs, the latter greatly enriched in brain, which arise from alternate polyA site usage. Similar *M2146l* mRNAs occur in mouse brain and heart, while human *M2146* expresses 5- and 1.9-kb transcripts, the latter enriched in brain and muscle. FISH using 4 BAC probes identifies the order tel-*Sra1*-*M2146*-*M2146l*-IC-cen in normal and Tg<sup>PWS</sup> mouse fibroblasts, with all 4 genetic loci deleted in Tg<sup>PWS</sup> mice. RT-PCR analysis with Tg<sup>PWS</sup> and Tg<sup>AS</sup> mouse brain and human PWS and AS imprinting mutations demonstrates that *M2146* and *M2146L* are expressed from both parental alleles, and hence are non-imprinted. Large duplicons map between non-imprinted and imprinted domains in human, with a 150-200 kb region with >80 kb composed of a high density of L1 repeats in mouse, which may form a boundary separating the 2 domains. Among alternative hypotheses, the Sp1 transcription factor (TF) can protect CpG islands from methylation, and as multiple consensus Sp1 sites occur in the promoters of the 2 new genes, Sp1 might protect from *de novo* methylation on the maternal allele and play a role in generating the non-imprinted/imprinted boundary.

**Large fragile X premutatin alleles may often contain two AGG interruptions.** *S.L. Nolin, G.E. Houck, Jr., A.D. Gargano, W.T. Brown.* Human Genetics Dept, NYS Inst Basic Research, Staten Island, NY.

Fragile X syndrome, a common cause of inherited mental retardation, is associated with a CGG repeat expansion within the FMR1 (fragile X mental retardation 1) gene. In the normal population, AGG triplets are usually present at regular intervals within the repeat region, most commonly at positions 10 and 20. AGG interruptions are thought to stabilize the region by preventing slippage during DNA replication. In families with fragile X, however, a different pattern of AGG interruptions has been observed. Several groups have used a variety of techniques to analyze the repeat organization in fragile X premutation males and observed either a single or no AGG interruption in the 5 end of the repeat and long tracts of uninterrupted CGG repeats in the 3 end. We have developed a procedure for sequencing the repeat that allowed us to examine the alleles in 20 premutation males with repeats ranging from 90 to 150. In contrast to the results of earlier studies, sequence analysis revealed that seven males had no AGG, seven had one AGG, and six had two AGG interruptions in the 5 end of the repeat. These results suggest that two AGG interruptions may be a common finding in males with large premutation alleles. If this proves to be the case in males, two AGG interruptions may also be observed in some premutation females, particularly in those whose offspring seem less likely to undergo expansion to a full mutation.

**Nuclear localization and dominant-negative suppression by a mutant SKCa3 N-terminal channel fragment identified in a patient with schizophrenia.** *M.J. Miller<sup>1</sup>, H. Rauer<sup>1</sup>, H. Tomita<sup>1</sup>, H. Rauer<sup>1</sup>, J.J. Gargus<sup>1,2</sup>, G.A. Gutman<sup>3</sup>, M.D. Cahalan<sup>1</sup>, K.G. Chandy<sup>1</sup>.* 1) Physiology and Biophysics, University of California, Irvine, Irvine, CA; 2) Division of Human Genetics, Department of Pediatrics, University of California, Irvine, Irvine, CA; 3) Department of Microbiology and Molecular Genetics, University of California Irvine, Irvine, CA.

The small-conductance calcium-activated K<sup>+</sup> channel gene SKCa3 / KCNN3 maps to 1q21, a region strongly linked to schizophrenia. Recently, a 4 bp deletion in SKCa3 was reported in a patient with schizophrenia. This frame-shift mutation would truncate the protein at the end of the N-terminal cytoplasmic region (SKCa3D). We generated a GFP-SKCa3 N-terminal construct (SKCa3-1/285) that is identical to SKCa3D except for the last two residues. Using confocal microscopy we demonstrate that SKCa3-1/285 localizes rapidly and exclusively to the nucleus of mammalian cells like several other pathogenic polyglutamine containing proteins. This nuclear targeting is mediated in part by two polybasic sequences present at the C-terminal end of SKCa3-1/285. In contrast, full-length SKCa3, SKCa2 and IKCa1 polypeptides are all excluded from the nucleus and express as functional channels. When over-expressed in human Jurkat T cells, SKCa3-1/285 can suppress endogenous SKCa2 currents, but not voltage-gated K<sup>+</sup> currents. This dominant negative suppression is most likely mediated through the co-assembly of SKCa3-1/285 with native subunits and the formation of non-functional tetramers. The nuclear localization of SKCa3-1/285 may alter neuronal architecture, and its ability to dominantly suppress endogenous SKCa currents may affect patterns of neuronal firing. Together, these two effects may play a part in the pathogenesis of schizophrenia and other neuropsychiatric disorders.



**A 33 CAG-repeat allele causes ataxia in a patient with a de novo mutation at the SCA2 locus.** *A.O. Rasmussen, M. Languren, M.E. Alonso-Vilatela, P. Yescas-Gómez.* Department of Neurogenetics, Instituto Nacional de Neurologia y Neurocirugia, Mexico City, Mexico.

The autosomal dominant spinocerebellar ataxias (ADCA) are a clinically and genetically heterogeneous group of disorders, caused by the expansion of unstable microsatellite repeats in several genes. Spinocerebellar ataxia type 2 (SCA2) is the most prevalent cause of ADCA in Mexican population, accounting for almost 50% of all cases. A large cohort of ataxia patients has been studied at the Instituto Nacional de Neurologia y Neurocirugia in Mexico City, including 75 sporadic cases. Among these, we identified a 40 year-old female with a very slowly progressive cerebellar ataxia (age at onset: 16) and negative family history. Clinical and laboratory information outruled acquired causes of ataxia, as well as Vitamin E-deficiency. Molecular analysis of the SCA2 gene demonstrated that she is a heterozygote for this locus with a normal 22-CAG repeat allele, and a 33 CAG-repeat allele. The analysis was extended to her parents, the father is a normal 22 CAG-repeat homozygote while her mother carries a long-normal allele. On clinical examination, both are healthy at the ages of 65 and 62 respectively. The proband has a 10 year old son whom she refused to be tested. This case supports a previous observation that SCA2 alleles with only 33 CAG repeats can be enough to cause disease, and the result of de novo expansions of longer-than-usual SCA2 alleles. Supported by CONACYT 37090-M.

**Screening of maternal uniparental disomy of chromosome 7 in pre- and postnatal growth retardation of unknown etiology.** *K. Hannula<sup>1</sup>, M. Lipsanen-Nyman<sup>2</sup>, P. Kristo<sup>1</sup>, I. Kaitila<sup>3</sup>, K.O.J. Simola<sup>5</sup>, H.L. Lenko<sup>6</sup>, P. Tapanainen<sup>7</sup>, C. Holmberg<sup>2</sup>, J. Kere<sup>1, 4</sup>.* 1) Dept Medical Genetics, Univ Helsinki; 2) Hospital for Children and Adolescents, Univ Helsinki; 3) Clinical Genetics Unit, Univ Helsinki; 4) Finnish Genome Center, Univ Helsinki; 5) Dept Clinical Genetic, Tampere Univ Hospital; 6) Dept Pediatrics, Univ Tampere; 7) Dept Pediatrics, Univ Oulu, Finland.

Many short-statured children lack an etiologic explanation for their growth retardation. Uniparental disomy (UPD), the inheritance of both chromosomes of a chromosome pair from only one parent, is associated with short stature for many chromosomes. Maternal UPD of chromosome 7 (matUPD7) is observed in 10% of Silver-Russell syndrome (SRS) cases. SRS is an extreme syndrome of intrauterine growth retardation (IUGR) and slight dysmorphic signs. MatUPD7 has also been reported in patients with only slight dysmorphic features and pre- or postnatal short stature. The aims of this study were to study the role of matUPD7 in growth failure of unknown etiology and SRS, and to evaluate the efficiency of testing for matUPD7 as a diagnostic tool. The patient cohort included 205 children with short stature of unknown etiology: 39 SRS cases, 91 patients with IUGR and subsequent postnatal short stature, and 75 patients with postnatal growth retardation (PNGR) only. MatUPD7 was screened for by genotyping DNA from the patient and parents with 13 chromosome-7-specific microsatellite markers. Six matUPD7 cases were observed (3%, 6/205) exclusively among 39 SRS patients (15%, 6/39). IUGR and/or PNGR patients with dysmorphic features did not reveal matUPD7 cases. All six matUPD7 patients have mild SRS signs and they have additional features not regarded as typical of SRS: excessive sweating, severe feeding difficulties, and speech delay. MatUPD7 cases seem to form a distinct entity among SRS cases. Our results indicate that matUPD7 is predominantly observed among SRS patients and matUPD7 is not a common cause for growth retardation. From a clinical perspective, screening of matUPD7 should be focused on patients with severe IUGR, features of SRS, speech difficulties, and poor feeding.

**Strong candidate region adjacent to D15S113 responsible for imprinting mechanism of Prader-Willi syndrome.**

*S. Ninomiya, Y. Yokoyama, C. Inoue, S. Yamashita, T. Une, M. Teraoka, Y. Seino.* Dept Pediatrics, Okayama Univ Graduate Sch of Medicine and Dentistry.

Prader-Willi syndrome (PWS) results from the loss of expression of one or more paternally expressed genes on human chromosome 15q11-q13. Most cases are due to a paternal deletion or maternal uniparental disomy (UPD) and rare cases have imprinting mutation. Here we report an atypical PWS case in which deletion was not detected but very small region, D15S113, CA repeat marker, had maternal hetero UPD. Clinical summaries: The patient, 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, bilateral convergent strabismus, bilateral second to fifth fingers skinny syndactyly, and cryptorchism. He had 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: The chromosomal analysis using high resolution banding by GTG banding method showed no deletion of 15q11-q13 and the fluorescent in situ hybridization (FISH) using a probe of SNRPN also revealed no deletion. Molecular analysis: Genomic DNA was extracted by the standard method. Highly polymorphic CA repeat markers within 15q11-q13 and other 15q regions were analyzed by PCR. Primer loci were D15S11, D15S128, D15S122, D15S210, D15S113, D15S1234, GABRB3, D15S165, D15S126, D15S153, D15S211 and D15S127. It demonstrated biparental inheritance at two loci within 15q11-q13 (D15S11 and D15S1234) and at two loci distal to 15q13 (15S126 and 15S211). Remarkable interestingly only one locus, D15S113, within 15q11.2-q12 showed maternal hetero UPD. The DNA methylation pattern of the patient using by SNRPN probe was normal. Conclusion: The result of partial maternal hetero UPD in this study suggests that a gene (genes) or an important unit of imprinting mechanism for PWS exists surrounding the narrow UPD region. In addition this suggests the UPD in very small region may be one of the cause of congenital multiple malformations.

**Molecular rescue by mitotic recombination as an explanation for recurrence of 11q- Jacobsen syndrome in two brothers.** *M.M. Haag<sup>1</sup>, L.S. Beischel<sup>1</sup>, C.L. McCann<sup>1</sup>, J.F. Reynolds<sup>1</sup>, S.M. Phillips<sup>1</sup>, M.L. Tunby<sup>1</sup>, J.C. Hansen<sup>2</sup>, J.P. Johnson<sup>1</sup>.* 1) Shodair Children's Hospital, Helena, MT; 2) Medical Associates, P.C., Bozeman, MT.

The 11q- syndrome (Jacobsen syndrome) involves characteristic but variable features including trigonocephaly, psychomotor retardation, cardiac anomalies, and thrombocytopenia. Most cases are de novo, resulting from deletions at 11q23 or distal. Inheritance of an expanded CCG repeat at the folate-sensitive fragile site, FRA11B, has been implicated in generation of the chromosome breakpoint in some Jacobsen syndrome patients. We describe the first family, to our knowledge, with recurrent 11q24-qter deletion in two brothers. Both presented at birth with thrombocytopenia and minor anomalies. The older brother was mosaic for an 11q- cell line (~10% normal). Molecular analysis shows that the breakpoints are near D11S4958 (11q23.3), distal to FRA11B, and that the deletion is identical and of maternal origin in both boys. Parental lymphocyte chromosomes were normal with no expression of a folate-sensitive fragile site under stressed culture conditions. However, mother is homozygous for all loci within the deleted region in her sons, and likely has uniparental disomy (UPD) for this region. We suggest that a grandparental chromosome has an 11q fragile site distal to FRA11B, and that mother experienced rescue from the 11q- syndrome by mitotic recombination. This resulted in uniparental disomy with tissue limited mosaicism for a deleted cell line to account for her two affected sons. We propose that the tissue limited rescue phenomenon also occurred in her mosaic son. The low level of normal cells in the older son did not allow molecular confirmation. The presumed post-zygotic segmental UPD in the mother may be another example of somatic recombination, a mechanism identified by loss of heterozygosity, as observed in certain cancers and in the segmental UPD 11p in Beckwith-Wiedemann syndrome. Here, we show this mechanism in recurrent Jacobsen syndrome. Other cases of familial recurrence of a chromosome abnormality in a syndrome may be candidates for this approach to identify mitotic recombination and UPD.

**Genetic Testing for BWS: Introduction of a Simple Quantitative Method for UPD Detection.** *C. Wei<sup>1</sup>, R. Weksberg<sup>2</sup>, L. Steele<sup>1</sup>, T.L. Stockley<sup>1</sup>, C. Shuman<sup>2</sup>, P.N. Ray<sup>1</sup>.* 1) Paediatric Laboratory Medicine, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Division of Clinical and Metabolic Genetics, Hospital for Sick Children, Toronto, Ontario, Canada.

Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome characterized by pre- and/or postnatal overgrowth, neonatal hypoglycemia, hemihyperplasia, abdominal wall defect and increased risk of childhood tumors. The imprinted region of chromosome 11p15.5 is involved in pathogenesis of BWS. Several molecular mechanisms have been identified including paternal uniparental disomy (UPD) of chromosome 11p15.5, paternal duplication or maternal translocation, imprinting defects at KvDMR1, and p57KIP2 mutation. Molecular diagnosis for BWS is optimal for patient management and accurate genetic counseling for the family. Paternal UPD of chromosome 11p15.5 is reported to account for approximately 20% of sporadic BWS cases. We have developed a multiplex PCR-based assay using microsatellite markers on chromosome 11 to detect paternal UPD in BWS patients. In 125 BWS probands tested, we detected chromosome 11p15 UPD in 21 cases, 16 of which were confirmed to be paternal in origin. Consistent with the literature, mosaicism was seen in all BWS patients with UPD, supporting the hypothesis that UPD arises at 11p15 from post-zygotic recombination between paternal and maternal chromosomes. The level of mosaicism detected varies between patients and specimen types (ie, whole blood, skin fibroblasts, and lymphoblasts). The lower limit of detection of UPD using this assay is 20% based on experimental variation detected in normal samples. In addition to UPD detection, this assay also detected 5 cases of paternal duplication of 11p15. Four of these cases showed two paternal alleles and one maternal allele at informative loci, indicating partial trisomy of 11p15 resulting from a paternal balanced translocation. One case with de novo 11p15 duplication was confirmed to be paternal in origin. In summary, we have shown that this quantitative multiplex PCR-based method is accurate and sensitive to detect paternal UPD as well as duplication of 11p15 in BWS patients.

**Methylation analysis of maternal and paternal uniparental disomy of human chromosome 14.** A.A. Wylie<sup>1</sup>, P.D. Cotter<sup>2</sup>, B. Williford<sup>3</sup>, P.R. Papenhausen<sup>3</sup>, R.L. Jirtle<sup>1</sup>. 1) Radiation Oncology, Duke University, Durham, NC; 2) Division of Medical Genetics, Childrens Hospital Oakland, CA; 3) Department of Cytogenetics, LabCorp Inc., Research Triangle Park, NC.

Abnormal phenotypes associated with uniparental disomy (UPD) have implied the presence of imprinted genes on a number of chromosomes. These include distinct clinical abnormalities associated with both maternal and paternal UPD of the long arm of human chromosome 14. The only imprinted genes identified to date on human chromosome 14 are the paternally expressed DLK1 and maternally expressed GTL2. As a first step in establishing if abnormal expression of DLK1 and/or GTL2 underlies the clinical phenotype observed in either maternal or paternal UPD14, we determined whether the DLK1/GTL2 imprinted locus is contained within the proposed chromosome 14 uniparental disomic region. A parent-of-origin dependent, differentially methylated region (DMR) lies immediately upstream of GTL2 and is proposed to regulate the imprinted expression of both DLK1 and GTL2. Using bisulfite sequencing for methylation analysis of this DMR, we determined the status of both the maternal and paternal alleles in normal controls and patients reported to have UPD14. Normal control samples analysed using this approach were differentially methylated and contained both methylated and unmethylated alleles. In contrast, paternal UPD samples contained only methylated alleles and maternal UPD samples contained only unmethylated alleles, consistent with their UPD status. These data indicate that for the samples analysed the DLK1/GTL2 locus is contained within the proposed region of uniparental disomy and abnormal expression of these genes may underly the clinical phenotype of maternal and/or paternal UPD14. In order to allow rapid analysis of future samples, a UPD14 methylation specific PCR (MSP) assay was also developed. This assay eliminates the need for parental DNA samples, does not require microsatellite marker analysis and will identify both maternal or paternal UPD in a single assay.

**Primary open-angle glaucoma GLC1A mutation testing in clinical practice.** *M.A. Aldred<sup>1,3</sup>, L. Baumber<sup>1,3</sup>, A. Hill<sup>2</sup>, K. Goh<sup>2</sup>, W. Karwatowski<sup>2</sup>, R.C. Trembath<sup>1,3</sup>.* 1) Leicestershire Genetics Centre, Leicester Royal Infirmary, Leicester, UK; 2) Department of Ophthalmology, Leicester Royal Infirmary, Leicester, UK; 3) Division of Medical Genetics, University of Leicester, Leicester, UK.

Primary open angle glaucoma (POAG) affects 1% of people over age 40. Early detection and treatment can prevent blindness, but the disease is often symptomless until a late stage. Positive family history is an important risk factor, but only ~50% of at-risk individuals take up free eye tests. Previous studies indicate that ~5% of POAG results from mutations in the GLC1A gene, raising the possibility of identifying individuals genetically predisposed to glaucoma. We collected DNA samples from 426 predominantly Caucasian POAG patients unselected for family history and sequenced exon 3 of GLC1A, where most mutations cluster. The Q368X mutation was found in 6 patients (1.4%). No other mutations were identified. It is unclear whether this reflects sampling error, or whether the prevalence of GLC1A mutations in this cohort is lower than in other populations. Genetic and glaucoma screening was offered to first-degree relatives of these 6 probands and of age/sex matched mutation-negative controls. Of 11 proband relatives, 3 carried Q368X, one of whom already had glaucoma. 8 were mutation negative, of which 2 were under treatment for glaucoma. Of 5 control relatives, all were mutation and glaucoma negative. We have thus identified 2 unaffected mutation carriers who may be at increased risk of developing glaucoma in later life. Upon review, the two mutation-negative relatives are not thought to have glaucomatous damage, but we caution against changing glaucoma surveillance regimens in such individuals.

**Identification of new FANCA mutations in Tunisian patients.** C. Bouchlaka<sup>1</sup>, K. Dellagi<sup>1</sup>, S. Abdelhak<sup>1</sup>, For the Tunisian Fanconi Anemia Study Group\*<sup>2,3,4,5,6</sup>. 1) Immunology Dept, Institut Pasteur, Tunis, Tunisia; 2) C.H.U.Aziza Othmana, Tunis; 3) C.H.U.Habib Bourguiba, Sfax; 4) C.H.U.Farhat Hached, Sousse; 5) CNGMO, Tunis; 6) Hopital de Kairouan.

Fanconi anemia (FA) is a genetically heterogeneous autosomal recessive rare disease. Five genes (FANCA, FANCC, FANCE, FANCG and FANCF) have been identified, and one (FANCD) have been solely localized. Forty patients belonging to thirty-one families from different geographical areas of Tunisia were investigated. Haplotype analysis and homozygosity mapping allowed us to assign Tunisian patients to FAA complementation group. Search for mutations was started by screening all the patients for the already described recurrent mutations (1263delF, 1115-1118del and 4267-4404del) found within FANCA in exons 13, 38 and 43 respectively. These mutations were identified in multiple unrelated patients from different populations. No one of these recurrent deletions nor other mutations were observed in these exons for our population. Screening for mutations in the remaining 40 exons of the FANCA gene and their flanking intronic sequences was performed using genomic DNA amplification and direct sequencing. Preliminary investigation allowed the identification of three novel mutations within FANCA gene. Two small homozygous deletions occurred in exon 17 (1693delT) and exon 19(1784-1788del) respectively resulting in a frame shift and a premature stop codon. These mutations may lead to the production of a truncated protein. The third mutation is an A to T transversion at position 166 within intron 24. This mutation leads to the production of an uncorrectly spliced mRNA with an insertion of 166 bp. Our results show the heterogeneity of the mutations affecting Tunisian FA patients.

\*2 to 6 - H.Ben Abid, B.Meddeb, Z.Belhaj Ali, R.Belakhal, L.Aissaoui, A.Hafsia (2) - M.Frikha, M.Elloumi, S.Mseddi, M.Hachicha, F.Fakhfakh, H.Ayadi(3) - S.Ennabli, H. Elomri, A. Amouri, H. Sennana, A.Saad (4). - T.Ben Othman, S.Ladeb, L.Tordjman, A.Ben Abdeladhim(5) - F. Amri(6).



**Variant patterns of intron 22 inversion in factor VIII gene in Indian hemophiliacs. *G.R. Chandak, M. Idris, K. Radha Mani.* Centre for Cellular Molecular Biology, HYDERABAD, Andhra Pradesh, India.**

**Hemophilia A is a common coagulation disorder resulting from an inherited deficiency of factor VIII. The disorder is inherited as X-linked recessive trait with a significant proportion of cases (20-30%) due to de novo mutations in the FVIII gene located at Xq28. Recurrent DNA inversion of intron 22 in the FVIII gene accounts for more than 40% of cases with severe Hemophilia-A. The inversion occurs between a region of intron 22 (int22h) and one of two homologous copies of this region (int22h-2 and int22h-3), located 300 to 400 kb telomeric to the FVIII gene. A BclI digestion of genomic DNA followed by Southern Hybridizations using p542.16 probe detects the disruption of factor VIII gene by showing a change in the characteristic three-band pattern (21.5 Kb, 16.0 Kb and 14.0 Kb bands). Different variants have been reported in the pattern of this inversion. We report 3 Indian families each with characteristic atypical pattern of inversion. All three patients had severe hemophilia-A, however their inhibitor status was not known. Family I showed an additional 23.5 Kb band whereas family II showed a 20 Kb band apart from the normal three bands. The normal pattern of 21.5 Kb, 16.0 Kb and 14.0 Kb was altered to 17.5 Kb, 16.0 Kb and 14.0 Kb in family III. Subsequent analysis using XbaI restriction fragment length polymorphism has shown variable copy number of int22h apart from the normal three copies. Further analysis has also been performed to analyse the associated inversion junction. The detailed analysis of these families in the light of these results will be presented. These patients and the variant inversions further stress on the association of int22h with Xq28 instability.**

**Molecular genetic analysis of Fanconi anaemia in Black South Africans.** A. Krause<sup>1</sup>, F. Essop<sup>1</sup>, L. Wainwright<sup>2</sup>, J. Poole<sup>3</sup>, C. Mathew<sup>4</sup>. 1) Department of Human Genetics, South African Institute for Medical Research, Johannesburg, South Africa; 2) Department of Paediatrics, Chris Hani Baragwanath Hospital, University of the Witwatersrand, Johannesburg, South Africa; 3) Department of Haematology and Oncology, New Johannesburg Hospital, University of the Witwatersrand, Johannesburg, South Africa; 4) Division of Medical and Molecular Genetics, GKT School of Medicine, Guy's Hospital, London, United Kingdom.

The Fanconi anaemias (FA) are a group of autosomal recessive genetic disorders characterised clinically by progressive bone marrow failure, skeletal deformities, early occurrence of malignancies and a predisposition to neoplasia. Spontaneous chromosome breakage is a feature of FA. Mutation screening is generally difficult because of marked genetic heterogeneity. In the South African Black population, the most common mutation is a 7bp deletion in exon 5 (637-643del) of the FA Complementation Group G gene. DNA analysis on Black patients with FA showed 85% to be homozygous for the FANCG mutation. This mutation is highly likely to have had a single origin, as evidenced by its tight association with the C allele of a SNP at IVS1+77 in the FANCG gene. The 7bp deletion was detected on 3/600 chromosomes from unaffected individuals, giving a carrier rate of 1/100. The birth rate for all FA in Black patients in SA is estimated to be 1/20 000, one of the highest rates in the world. The majority (19/20) of these cases are still not being diagnosed. The presence of a single common mutation allows for rapid diagnostic testing in this group, with possibilities for preclinical, prenatal and carrier testing in the majority of Black South African families.

**Correlation of SMN and NAIP gene deletions with the phenotype of Spinal Muscular Atrophy in Indian patients.**  
*M. Idris<sup>1</sup>, M. Nair<sup>2</sup>, G.R. Chandak<sup>1</sup>.* 1) Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India; 2) Shri Chitra Thirunal Institute of Medical Sciences, Trivandrum, Kerala, India.

The spinal muscular atrophies (SMAs) form a heterogeneous group of diseases inherited as autosomal dominant, autosomal recessive, or X linked recessive traits. Three candidate genes have been identified and shown to be deleted in SMA patients: the Survival Motor Neuron gene (SMN), the Neuronal Apoptosis Inhibitory Protein gene (NAIP) and the XS2G3 cDNA. In this report, we present the molecular analysis of the SMN exons 7 and 8 & NAIP exons 5 and 13 in 16 Indian patients with a clinical diagnosis of SMA. Exons 7 and 8 of SMN were homozygously deleted in all 10 SMA type I patients, three Type II patients and one out of three Type III patient. Exon 5 of NAIP gene was homozygously deleted in seven Type I patients only. None of the type II and III patients had deletion in exon 5 and 13 of NAIP gene. In an attempt to establish the genotype-phenotype correlation, it was noted that all SMA type I patients with a deletion in both SMN and NAIP had serious breathing difficulties and expired during the first six months of life whereas the remaining with a deleted SMN but intact NAIP needed respiratory support only after the first year of life. Homozygous deletion of exon 5 of NAIP was detected in a fetus during prenatal diagnosis in a couple. They had an earlier child who expired at the age of 6 months with a clinical diagnosis of type I disease. However, no deletion of SMN gene at exons 7 and 8 and exon 13 of NAIP gene was noted. None of the parents showed homozygous deletion of exon 5 in the NAIP gene. Q-PCR analysis did show variable copy number in the parents but no deletion of any copy of NAIP gene could be detected. This led to diagnostic dilemma and brings in ethical and social issues. The present findings support the hypothesis that SMN deletion plays an important role in the development of SMA and suggest that combined deletion of both SMN and NAIP may be relevant for determining the disease severity. However, the role of isolated NAIP deletion in the causation of phenotype of SMA may need to be investigated.

**Connexin 26 mutations in Iranian patients with autosomal recessive nonsyndromic sensorineural hearing loss.** *S. Sahebjam<sup>1</sup>, N. Kouchakian<sup>1</sup>, S. Arzhangi<sup>1</sup>, K. Kahrizi<sup>1</sup>, M. Menzens<sup>2</sup>, R.J.H. Smith<sup>2</sup>, H. Najmabadi<sup>1</sup>.* 1) Genetics Research Center, University of Welfare Sciences, Tehran, Tehran, Iran; 2) Molecular Otolaryngology Research Laboratories, Department of Otolaryngology Head and Neck Surgery, University of Iowa; Iowa, IA United States.

Hereditary hearing loss is an extremely common disorder. About 70% of hereditary deafness is non-syndromic. Autosomal recessive forms make up about 85% of these cases. Many deafness genes exist, but the most common cause of hereditary hearing loss in many different populations is the mutations of connexin 26 (GJB2), which mainly lead to autosomal recessive non-syndromic hearing loss (ARNSHL). This study is aimed to investigate the mutations of connexin 26 gene (GJB2); found in patients with autosomal recessive nonsyndromic sensorineural hearing loss in the Iranian population. Fifty-five unrelated patients were studied and GJB2 mutations were found in 22 (20%) chromosomes with the 35delG mutation standing first. R127H, W24X, and R184P were the other GJB2 mutations detected. The relative frequency of connexin 26 mutations is much less than reports of other populations. This raises the possibility that mutations located in other loci be the major cause of ARNSHL in Iranian patients.

**Variable phenotype in monozygotic twins with Duchenne Muscular Dystrophy. *K. Radha Mani, G.R. Chandak.* Centre for Cellular and Molecular Biology, Hyderabad, Andhra Pradesh, India.**

Duchenne and Becker Muscular Dystrophy are X-linked recessive allelic neuromuscular disorders where affected individuals typically have pseudohypertrophy of calf muscles with development of secondary atrophy and contraction and cardiac or respiratory muscle involvement in the later stages. The disease is caused by mutation in the DMD gene and approximately 65% of patients have intragenic deletions, the rest have point mutations or duplications. We report an interesting case of monozygotic twins with variable phenotype on a similar genetic background. The patients presented to us at the age of 12 years, with one of them almost immobilized with an age of onset at 4 years (Twin I) whereas the other fairly mobile with an age of onset only at the age of 8 years (Twin II). Although both had similar pseudohypertrophy of the calf muscles but the twin I had comparatively flabby muscles consistent with degenerating muscles. The CPK levels were 28,000 and 13,000 respectively for twin I and twin II. Cytogenetic study for both was consistent with a male karyotype with a normal banding pattern. DNA fingerprinting using a 10-loci microsatellite showed similar profile and matched with the parents thus confirming paternity. Deletion analysis of the DMD gene showed exon 49-50 deletion out of 25 exons analysed. Analysis of entire DMD gene using cDNA probes failed to show any other missing or abnormal restriction fragment. Both twins showed deletion of 1.6 Kb and 3.7 Kb fragments on a HindIII blot hybridized with cDNA 8 of the DMD gene. Neither the mother nor the relatives showed this deletion suggesting the deletion to be a new mutation in this pair of monozygotic twin. Two possible explanations can be advanced to account for this variability on a seemingly similar genetic background. This may be an evidence for somatic mosaicism or it may suggest the presence of other factors modulating the severity of the DMD phenotype.

**Homozygosity for the V37I Connexin 26 mutation in 3 unrelated children of Asian background with sensorineural hearing loss.** *P.M. Fortina<sup>1</sup>, L. Bason<sup>2</sup>, T. Dudley<sup>3</sup>, K. Lewis<sup>3</sup>, U.K. Shah<sup>4</sup>, W. Potsic<sup>4</sup>, I.D. Krantz<sup>2</sup>.* 1) Division of Clinical Pathology; 2) Division of Human Genetics and Molecular Biology; 3) Division of Audiology; 4) Division of Otolaryngology, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Mutations in Connexin 26 (Cx26) account for approximately 20% of all childhood deafness and approaches 50% in documented recessive cases of sensorineural hearing loss (SNHL). Two mutations in particular, 35delG and 167delT, account for more than 90% of reported mutations in this gene and several mutations predominate in specific ethnic populations (167delT in Ashkenazi Jews and 235delC in Japanese individuals). The V37I amino acid change was initially reported as a polymorphism by Kelley et al (*Am J Hum Genet* 62:792-799, 1999) when it was seen in a heterozygous state in a sample from a control group. Subsequently Rabionet et al (*Hum Genet* 106:40-44, 2000) found the same change in a homozygous state in an individual with hearing loss and not in 100 control samples. All individuals tested in the Rabionet study were described as "Spanish" or "Italian", while the ethnic background of the individual reported by Kelley et al was not specified. We have identified 3 unrelated individuals homozygous for the V37I mutation with sensorineural hearing loss. Patient 1 has a 40-50 dB bilateral SNHL with increase in severity in the higher frequencies, patient 2 had a 55-60 progressing to 70-100 dB bilateral SNHL over 4 years (from 2-6 years), and patient 3 has a bilateral high frequency hearing loss starting at 2000 Hz and increasing to 40-60 dB at 4000 Hz. Patient 2 is the oldest of the 3 patients. It is unclear if this mutation predisposes to progression of hearing loss. One individual is of Philippine ancestry, and the other 2 are of Chinese background, raising the possibility that this mutation may be more frequent amongst Asian populations. Further screening of controls of Philippine and Chinese background is being undertaken to determine the carrier status of this mutation in these populations.

**Long QT syndrome:reduced penetrance in heterozygotes.** *H.E. Hughes<sup>1</sup>, A. Murray<sup>2</sup>, L. Emmerson<sup>1</sup>, R. Evans<sup>1</sup>, W. Reardon<sup>3</sup>, S. Jeffery<sup>2</sup>.* 1) Institute of Medical Genetics, Univ Hosp Wales, Cardiff, Wales; 2) Medical Genetics Unit, St. George's Hospital, London; 3) National Centre for Medical Genetics, Dublin, Ireland.

Long QT syndrome is recognized as a genetically heterogeneous disorder characterized by syncopal episodes. A number of different loci and gene mutations have been delineated - the first being KVLQT1, also known as KCNQ1. The phenotype usually is expressed in the heterozygous state (Romano-Ward syndrome). In the one well-recognized recessive phenotype (Jervell and Lange-Nielsen syndrome), caused by a homozygous mutation in the above gene, arrhythmia is associated with deafness. We report a large kindred where the proband, the product of a first cousin marriage, presented with repeated syncopal attacks at the age of 4 years. Both the proband and her asymptomatic brother, now aged 16 years, were shown to have prolonged QT interval and molecular analysis revealed a homozygous G269S mutation in exon 5 of the KVLQT1 gene. The proband, now deceased, was not known to have a hearing problem and audiometric testing of the brother shows no evidence of a hearing loss. Molecular analysis of extended family members over three generations has identified 8 other heterozygous carriers, the eldest of whom is 81 years of age. Six of these individuals, as well as the homozygous brother, have a significantly prolonged QT interval but none has a history of recurring syncope. Of the six heterozygotes who have had audiometric testing, four have normal hearing. However, other factors, such as industrial exposure and the possibility of another dominant gene, influence the development of hearing loss in this family. Two heterozygotes and other relatives who have tested negative for the above mutation have documented audiometric abnormalities. Reduced penetrance in heterozygotes in this family supports the suggestion that gene mutations for long QT syndrome are more common in the population than has been appreciated to date. This has implications for counselling and management of identified families. Furthermore, the family provides additional evidence that a homozygous mutation in this gene is not necessarily associated with a significant hearing loss in childhood.

**Mutations in the Cx26 and CDH23 gene cause indistinguishable profound congenital hearing loss in a large Dutch family.** *H. Kremer<sup>1</sup>, P. Van Hauwe<sup>1,2</sup>, L.H. Hoefsloot<sup>2</sup>, A. de Brouwer<sup>1,2</sup>, R. Pennings<sup>1</sup>, A. Deutman<sup>3</sup>, L.M. Astuto<sup>4</sup>, W.J. Kimberling<sup>4</sup>, J.M. Bork<sup>5</sup>, C.W.R.J. Cremers<sup>1</sup>, F.P.M. Cremers<sup>2</sup>.* 1) Dept. Otorhinolaryngology, UMC Nijmegen, Nijmegen, The Netherlands; 2) Dept. Human Genetics, UMC Nijmegen, Nijmegen, The Netherlands; 3) Dept. Ophthalmology, UMC Nijmegen, Nijmegen, Netherlands; 4) Gene Marker Laboratory, Boystown National Research Hospital, Omaha, NE USA; 5) Laboratory of Molecular Genetics, NIH, Rockville, MD USA.

In 1989, we ascertained a large family with apparent autosomal recessive non-syndromic childhood deafness. A genome wide scan with 300 polymorphic markers was not successful. Later, analysis of candidate loci for DFNB revealed genetic heterogeneity. In one branch (A) of the family the 35delG mutation in the Cx26 gene is homozygously present. In two other branches (B and C) the DFNB12 locus is involved. Testing patients of the branches B and C for mutations in the CDH23 gene revealed that the D2148N mutation is homozygously present in branch B and heterozygously in branch C. The D2148N mutation was not present in 100 control individuals but was detected heterozygously in another DFNB12 family corroborating its pathogenic nature. Further analysis of the CDH23 gene in order to find the second mutation in branch C is ongoing. Audiometric evaluation of the Cx26-related and CDH23-related hearing loss revealed no significant differences. Since mutations in the CDH23 gene also can cause USH1D with a variable retinal phenotype the patients with the CDH23 mutation will undergo ophthalmologic examination in order to contribute to the phenotype-genotype correlation of mutations in the CDH23 gene. Although the pedigree of the family initially suggested that it was ideal for the identification of a locus involved in autosomal recessive deafness, the data presented here illustrate the pitfalls in studying genetically heterogeneous hereditary hearing loss.



**Genetic Evidence for Digenic Inheritance of Non-Syndromic Deafness Involving the Connexin 26 Locus.** *W.E. Nance<sup>1</sup>, M. Tekin<sup>1</sup>, K.O. Welch<sup>2</sup>, X.J. Xia<sup>1</sup>, X.Z. Liu<sup>1</sup>, A. Pandya<sup>1</sup>, K.S. Arnos<sup>2</sup>*. 1) Dept Human Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Dept Biology, Gallaudet University, Washington, DC.

Mutations in the *GJB2* (Connexin26/Cx26) gene are known to account for greater than 50% of recessive non-syndromic deafness in certain populations. More than 50 different mutations in the single coding exon of the Cx26 gene have been reported. However, a substantial number of deaf individuals (1-10% of the screened deaf population) have been reported to carry a single heterozygous mutation in this gene. Mutations in the regulatory region of Cx26 or a second gene have been suggested as the cause for hearing loss. Screening for Cx26 mutations in a nationwide repository of deaf probands revealed two families, of the type shown in Fig.1, in whom the Cx26 mutations were not sufficient to explain deafness in all individuals. A second deafness gene, interacting with Cx26, has remained the most parsimonious explanation for hearing loss in these individuals. Screening for mutations in the Cx30 and Cx31 has been negative to date. We are continuing our search for mutations in other genes causing NSDF. The family structures and our data strongly support the hypothesis that Cx26 interacts with a second deafness gene. Now that an increasing number of specific forms of genetic deafness can be identified by molecular techniques, it is apparent that the analysis of marriages among the deaf will provide a previously unexploited technique for identifying epistatic interactions among genes for deafness. No matter how rare they are, assortative mating tends to amplify the frequency of genes that interact with Cx26, just as this mechanism specifically increases the frequency of Cx26 deafness itself.

**Molecular study of connexin 31 gene in nonsyndromic hearing impairment.** *E. Sartorato*<sup>1</sup>, *F. Alexandrino*<sup>1</sup>, *A. Maciel-Guerra*<sup>2</sup>. 1) Laboratorio de Genetica Humana - CBMEG, UNICAMP, Campinas - SP, Brazil; 2) Departamento de Genetica Medica - FCM, UNICAMP, Campinas - SP, Brazil.

Congenital deafness occurs in approximately 1 in 1000 live births. In developed countries half of this hearing loss is genetic. However, in Brazil the majority of cases of hearing loss are due to environmental factors, such as congenital infections (mainly rubella), perinatal anoxia, kernicterus and meningitis. There has been enormous progress in nonsyndromic deafness research during the last five years, with the identification of over 70 loci, and 19 genes. Among these, three connexin genes are related to either autosomal dominant or recessive hearing impairment (GJB2, connexin 26; GJB6, connexin 30; GJB3, connexin 31). Mutations in GJB2 account about 50% of all congenital cases of hearing impairment. The analysis of deaf patients lead to the identification of a missense mutation in a family with dominant hearing impairment in the connexin 30 gene. Mutations in GJB3, connexin 31 (Cx31) have been associated with a dominant and recessive form of deafness, as well as, several cases of erythrokeratoderma variabilis (EK). Eighteen nonsyndromic hearing loss families without mutations in the GJB2 were screened for mutations in the GJB3 gene. Besides other alterations a novel dominant mutation in GJB3 was found in a patient with pre-lingual severe deafness. Only two dominant mutations in the GJB3 gene were described before in families characterized by bilateral high-frequency hearing impairment.

**Mutations in the TMPRSS3 gene are a rare cause of childhood non-syndromic deafness in Caucasian patients.** *M. Wattenhofer<sup>1</sup>, S. Alwan<sup>1</sup>, C. Rossier<sup>1</sup>, R. Rabionet<sup>2</sup>, B. Montserrat-Sentis<sup>2</sup>, M.L. Arbones<sup>2</sup>, X. Estivill<sup>2</sup>, A. Pampanos<sup>3</sup>, T. Iliades<sup>3</sup>, M.B. Petersen<sup>3</sup>, M.V. Di Iorio<sup>4</sup>, A. Pasquadibisceglie<sup>4</sup>, M. D'Amelio<sup>4</sup>, P. Gasparini<sup>4</sup>, H-H.M. Dahl<sup>5</sup>, L. Dougherty<sup>6</sup>, H.S. Scott<sup>6</sup>, S.E Antonarakis<sup>1</sup>.* 1) Medical Genetics, University of Geneva School of Medecine, CH; 2) Medical & Molecular Genetics Center, Institut de Recerca Oncologica: Barcelona, Sp; 3) Institut of Child Health: Athens, Gr; 4) Medical Genetics Service, IRCCS-CSS: Rotondo, I; 5) The Murdoch Institute, Royal Children's Hospital, Melbourne, Aus; 6) Genetics and Bioinformatics Division, WEHI, Melbourne, Aus.

About 50% of early-onset deafness cases are genetic and 50% of the recessive cases are due to mutations in the GJB2 gene. The TMPRSS3 gene which encodes a potential transmembrane serine protease, is mutated in the families used to describe both the DFNB10 and DFNB8 loci on chromosome 21 (Scott et al., Nat Genet:27, 59-63, 2001). To determine if TMPRSS3 mutations are an important contributor to the etiology of childhood deafness, we performed SSCP screening of all 13 exons and intron-exon junctions and/or sequencing of DNAs of non-syndromic recessive deaf patients negative for GJB2 gene mutations. A total of 455 such unrelated patients (99 Spanish, 198 Italian, 93 Greek and 65 Australian) were enrolled in the study. Pathogenic mutations have been found in only two patients. A deletion of nucleotide 207C in exon 4 has been found in homozygosity in one Spanish patient resulting in a truncation of the TMPRSS3 protein after the transmembrane domain. A Greek patient is compound heterozygote for del 207C and D103G substitution. D103 is located in the LDLR domain and is well conserved in all examined LDLR domains. In addition, several common known exonic polymorphisms were found (V53I, G111S, I253V) as well as a series of intronic changes. Two new exonic polymorphisms that change amino acids have also been found: D173N and A426T. These results indicate that mutations in TMPRSS3 do not substantially contribute to the non-syndromic deafness in the Caucasian population. The frequency of TMPRSS3 mutations in our sample is 0,44% and is estimated to be 0,2 % in the general Caucasian childhood deafness population.

**The incidence of GJB2 mutations in prelingual deafness in the Greek population.** A. Pampanos<sup>1</sup>, T. Iliades<sup>2</sup>, N. Voyiatzis<sup>2</sup>, N. Eleftheriades<sup>2</sup>, V. Iliadou<sup>2</sup>, J. Economides<sup>3</sup>, P. Leotsakos<sup>3</sup>, P. Neou<sup>4</sup>, M. Tsakanikos<sup>4</sup>, L. Katsichti<sup>4</sup>, T. Antoniadis<sup>5</sup>, I. Konstantopoulou<sup>6</sup>, D. Yannoukakos<sup>6</sup>, K. Gronskov<sup>7</sup>, K. Brondum-Nielsen<sup>7</sup>, M. Grigoriadou<sup>1</sup>, J. Gyftodimou<sup>1</sup>, A. Skevas<sup>8</sup>, M.B. Petersen<sup>1</sup>. 1) Dept Genetics, Inst Child Hlth, Athens, Greece; 2) Aristotle Univ of Thessaloniki, Greece; 3) "Aghia Sophia" Children's Hosp, Athens, Greece; 4) "P. & A. Kyriakou" Children's Hosp, Athens, Greece; 5) "MITERA" Matern Surg Center, Athens, Greece; 6) N.C.S.R. "Demokritos", Athens, Greece; 7) JF Kennedy Inst, Glostrup, Denmark; 8) Univ of Ioannina, Greece.

Mutations in the gene encoding the gap-junction protein connexin 26 (GJB2) on chromosome 13q11 have been shown as a major contributor to prelingual, sensorineural, non-syndromic deafness. One specific mutation, 35delG, has accounted for the majority of the mutations detected in the GJB2 gene in Caucasian populations. A carrier frequency of the 35delG mutation of 3.5% has previously been reported in the Greek population. In a collaboration with the major referral centers for childhood deafness in Greece, patients were examined by an extensive questionnaire to exclude syndromic forms and environmental causes of deafness and by allele-specific PCR for the detection of the 35delG mutation. When syndromic forms and environmental cases were excluded, the 35delG mutation was found in 48.6% of the chromosomes in 36 familial cases (16 homozygotes and 3 heterozygotes) and in 32.4% of the chromosomes in 111 sporadic cases (32 homozygotes and 8 heterozygotes). Patients heterozygous for the 35delG mutation were analyzed by direct genomic sequencing of the coding region of the GJB2 gene, which revealed the W24X (2 alleles), L90P (2 alleles), 291insA (1 allele), and R184P (1 allele) mutations. In 5 35delG heterozygotes, no second GJB2 mutation could be detected. We conclude that GJB2 mutations are responsible for a large proportion of prelingual, non-syndromic deafness in the Greek population, and that allele-specific PCR is an easy screening test for the common 35delG mutation.

**Pendred syndrome mutations in the PDS gene cause a loss of iodide transport function and protein**

**mislocalisation.** *J.P. Taylor<sup>1</sup>, R. Metcalfe<sup>2</sup>, A.P. Weetman<sup>2</sup>, P.F. Watson<sup>2</sup>, R.C. Trembath<sup>1</sup>.* 1) Genetics, University of Leicester, Leicester, Leicestershire, United Kingdom; 2) Division of Clinical Sciences, University of Sheffield, Sheffield, United Kingdom.

Pendred syndrome is an autosomal recessive disorder with a variable phenotype, classically characterised as the association between hearing loss and thyroid goitre. This syndromic form of deafness is caused by mutations in the *PDS* gene, encoding an iodide/chloride transporter protein. Pendrin is located at the apical membrane of the thyroid follicle cells where it is hypothesised to be responsible for iodide efflux into the colloidal space. It is also expressed within distinct regions of the inner ear, although its role here is yet to be defined.

Over 50 mutations located throughout 18 of the 20 coding exons, have been reported and associated with a wide spectrum of phenotypes. To aid understanding of the genotype-phenotype correlation we studied the molecular mechanism by which 8 *PDS* mutations, previously identified within our patient cohort, relate to thyroid dysfunction in Pendred syndrome. The ability of wild-type and mutant pendrin to efflux iodide was assayed in HEK293 cells transiently transfected with GFP-tagged *PDS* constructs and NIS, the sodium iodide symporter expressed in the thyroid and responsible for iodide influx. All mutations studied caused a complete or partial loss of transport function in this system. At a cellular level, localisation studies of the mutant pendrin proteins revealed that some fail to reach the cell membrane and are, instead, retained within the endoplasmic reticulum. Mutations leading to a non-functioning, mislocalising protein are associated with a variable patient phenotype, indicating that genetic background and or environmental factors play a significant role in determining the extent of the clinical disorder. Of particular interest, the mutations 1337A>G and 1229 C>T both showed complete loss of iodide transport function, yet in the homozygous state in humans are associated with normal thyroid function, confirming a level of redundancy for pendrin within the thyroid gland.

**The osteogenesis imperfecta mouse (*oim*) thoracic aorta has decreased biomechanical integrity associated with reduced collagen content and increased pyridinoline crosslinks.** C.L. Phillips<sup>1</sup>, B.J. Pfeiffer<sup>1</sup>, R.A. Bank<sup>2</sup>, C.L. Franklin<sup>3</sup>. 1) Dept Biochemistry/Child Health, Univ Missouri, Columbia, MO; 2) TNO Prevention & Health, Leiden, The Netherlands; 3) Dept Veterinary Pathobiology, Univ Missouri, Columbia, MO.

Molecular defects in type I collagen (COL1A1 or COL1A2 genes) can impact mineralized as well as non-mineralized tissues. The primary load bearing components of the aorta are elastin and collagen. Using the osteogenesis imperfecta model mouse [*oim*, homozygous null for a COL1A2 gene, synthesizing only homotrimeric type I collagen,  $[a1(I)]_3$ , instead of normal heterotrimeric type I collagen,  $[a1(I)]_2 a2(I)$ ] we demonstrated that homozygote *oim* mouse aortas have significantly reduced breaking strength and decreased stiffness (incremental elastic modulus) as compared to wildtype (+/+) mouse aortas, with the greatest effect circumferentially and in the descending thoracic aorta. To determine if the decreased biomechanical integrity is associated with alterations in collagen content and/or collagen crosslinking we quantitated total collagen and non-reducible pyridinoline crosslinks in aortic tissues. Both ascending and descending portions of the *oim* thoracic aorta had significantly reduced collagen content relative to +/+ ascending and descending aortas. Yet, the ascending *oim* aorta had significantly *increased* levels of the non-reducible crosslink, hydroxylysylpyridinoline (H-Pyr) [ $0.541 \pm 0.036$  H-Pyr/collagen molecule] as compared to +/+ ascending aorta [ $0.365 \pm 0.032$  H-Pyr/collagen molecule,  $p = 0.005$ ]. The descending *oim* aorta also had increased H-Pyr as compared to +/+ descending aorta, though it was not significant,  $p = 0.077$ . The non-reducible crosslink, lysylpyridinoline, was not detectable. These findings suggest that the absence of pro $\alpha 2(I)$  collagen chains significantly weakens the *oim* thoracic aorta and that the reduced biomechanical properties of the *oim* aorta maybe due in part to decreased collagen content. Moreover, these studies suggest a potential compensatory protective mechanism via increased collagen crosslinking that may stabilize the vascular matrix when there is reduced and/or abnormal type I collagen present.

**Linkage disequilibrium suggests a common origin for Middle Eastern populations of connexin 26 (GJB2) mutations leading to deafness.** *H. Shahin*<sup>1, 2</sup>, *T. Walsh*<sup>3</sup>, *T. Sobe*<sup>2</sup>, *M-C. King*<sup>3</sup>, *K.B. Avraham*<sup>2</sup>, *M. Kanaan*<sup>1</sup>. 1) Dept of Life Sciences, Bethlehem Univ, Palestinian Authority; 2) Dept of Human Genetics and Molecular Medicine, Tel Aviv Univ, Israel; 3) Depts of Medicine and of Genomic Sciences, Univ Washington, Seattle WA.

Mutations in GJB2 are the most frequent known cause of genetic deafness. In most populations, the most common GJB2 mutant alleles are 35delG and 167delT. To explore the origins of these mutations and the historic demography of the Middle Eastern populations in which they occur, we identified SNPs 5-prime of GJB2 (-3558 T/C) and 3-prime of GJB2 (+1749 T/C, relative to ATG=+1 on genomic sequence). The SNPs are separated by 5.3 kb. We genotyped unrelated Palestinian and Israeli deaf probands with known GJB2 mutations and Palestinian and Israeli hearing controls. Allele frequencies among hearing controls at (-3558) were 0.86/0.14 in Israelis and 0.74/0.26 in Palestinians and at (+1749) were 0.88/0.12 in both populations. The most frequent (-3558)(+1749) haplotype, TT, had frequencies of 0.65 among Palestinian and 0.74 among Israeli hearing controls.

GJB2 167delT occurred exclusively on the TT haplotype in both populations. GJB2 35delG also occurred most frequently on the same haplotype in both populations. However, the consensus 35delG haplotype (C at -3558 and T at +1749) was relatively uncommon among both Palestinian and Israeli hearing controls (frequencies 0.27 and 0.14, respectively). Linkage disequilibrium (LD) measured by the Yule coefficient over 3.6 kb between the 5-prime SNP and 35delG was 0.83 among Palestinians and 0.82 among Israelis; LD over 1.7 kb between 35delG and the 3-prime SNP was 1.0 in both populations. The observations that the consensus 35delG haplotype was (a) the same in the two deaf populations and (b) relatively rare in each suggest that GJB2 35delG occurred once in a population ancestral to present communities in the Middle East. Similar LD values among Palestinians and Israelis between sites of deafness-associated GJB2 mutations and adjacent SNPs further support the antiquity and common shared origin of the mutant alleles.

**Low Frequency and Unique Spectrum of Connexin 26 (Cx26) Mutations in Mongolia: Role of Relaxed Selection and Assortative Mating.** A. Pandya<sup>1</sup>, X.J. Xia<sup>1</sup>, R. Erdenetungalag<sup>2</sup>, M. Tekin<sup>1</sup>, T.W. White<sup>3</sup>, J. Radnaabazar<sup>2</sup>, B. Dangaasuren<sup>2</sup>, W.E. Nance<sup>1</sup>. 1) Dept Human Genetics, Medical Col Virginia, Richmond, VA; 2) Dept Human Genetics and Human Reproduction, Maternal and Child Health, Ulaanbaatar, Mongolia; 3) State Univ New York, Stony Brook, NY.

Cx26 mutations account for 20-40% of all prelingual deafness. Heterosis, founder effects and mutation hot spots have been suggested to explain the high frequency of Cx26 deafness. Alternatively, we have proposed that Cx26 deafness may have been specifically amplified during the past 200-300 years by relaxed selection and assortative mating in some but not all parts of the world. To search for such correlations, we measured the frequency of Cx26 deafness in 316 deaf probands, and obtained measures of the genetic fitness and marriage patterns in 160 deaf adults from Mongolia. Since there is no "Deaf culture" or a long tradition of intermarriage among deaf in Mongolia, we expected the genetic fitness, assortative mating and frequency of Cx26 deafness to be low in this country. A survey of 160 deaf adults from Ulaanbaatar city revealed an assortative mating rate of 0.375, much lower as compared to 0.86 in the USA. An overall genetic fitness rate of 0.6 was observed compared to 0.8 for deaf in the USA. Of the 316 probands screened for mutations at the Cx26 locus, 43 had bi-allelic changes. However, only 4 carried both mutations that were definitely pathogenic, accounting for 2-4% of deafness in Mongolia, which is significantly lower than in Caucasian populations. 235delC was the most prevalent mutation (1.6% of alleles), and 35delG was only noted as a heterozygous change in 3 probands (0.5% of alleles). 39 probands had the V27I + E114G double mutation occurring in cis, with 3 being homozygous for double mutation. In vitro functional studies in a xenopus oocyte system revealed loss of intercellular conductance to  $\approx$  95% of WT constructs suggesting a pathogenic role for the double mutant. However, the presence of this change in one normal control raises issue about its clinical relevance role in causing hearing loss.



**Candidate screening for non-syndromic recessive auditory neuropathy.** *R.J. Rogers<sup>1</sup>, P.M. Kelley<sup>1</sup>, B.J.B. Keats<sup>2</sup>, A. Starr<sup>3</sup>, K. Kirschhofer<sup>4</sup>, S.M. Leal<sup>5</sup>, E.S. Cohn<sup>1</sup>, W.J. Kimberling<sup>1</sup>.* 1) Center for Hereditary Communication Disorders, Boys Town National Research Hospital, Omaha, NE 68131; 2) Department of Genetics, LSU Health Sciences Center, New Orleans; 3) Department of Neurology, University of California, Irvine; 4) Department for Oto-Rhino-Laryngology, University of Vienna, Vienna, Austria; 5) Laboratory of Statistical Genetics, The Rockefeller University, New York.

Auditory neuropathy (AN) is a type of hearing loss characterized by absent or abnormal auditory brainstem response with normal outer hair cell function. These individuals generally have poor speech reception out of proportion to the degree of hearing loss and have disappointing results with hearing aids. Most AN cases have an associated peripheral neuropathy, however there is a non-syndromic form which is less common and inherited as an autosomal recessive. In order to find the gene responsible for a type of non-syndromic recessive auditory neuropathy (NSRAN), a genome screen was initiated and linkage analysis was performed on a group of four NSRAN families using LINKAGE program version 5.1 (Lathrop et al. 1984). LOD scores were generated using MLINK for 2-point analyses and LINKMAP for multipoint analyses. Multipoint linkage analysis produced a maximum LOD score of 3.47, while 2-point linkage analysis gave a maximum LOD score of 2.908. Screening of a candidate gene revealed mutations, which we believe are pathologic. Since approaches to treatment and remediation differ between AN and non-AN groups, these findings have important implications for diagnosis, newborn screening, and prognostication. This study emphasizes that audition is a complex phenomenon whose processes can be dissected by modern methods of audiologic analysis.

**Identification and functional analysis of amino acid variants in the connexin26 (GJB2) gene.** *R. Rabionet<sup>1</sup>, E. Thönnissen<sup>2</sup>, A. Bosch<sup>1</sup>, B. Montserrat-Sentís<sup>1</sup>, N. López-Bigas<sup>1</sup>, A. Borragán<sup>3</sup>, M.L. Arbonés<sup>1</sup>, K. Willecke<sup>2</sup>, X. Estivill<sup>1</sup>.* 1) CGMM, Cancer research institute, Avia Castelldefels, Km 2.7, Barcelona, Spain; 2) Institut für Genetik, Universität Bonn, Römerstr. 164, 53117 Bonn, Germany; 3) Centro de Foniatría y Logopedia, Santander, Spain.

Frameshift mutations are the most common defects in the connexin26 gene (GJB2) causing congenital deafness. A large number of amino acid changes have been described, some leading to autosomal dominant hearing loss (W44S, W44C, and I20T). Other amino acid changes cause syndromic hearing impairment with skin disease and autosomal dominant transmission (G59A, D66H). A large number of changes have been detected in patients that have recessive hearing impairment (Connexin-Deafness Homepage <http://www.iro.es/deafness>). The functional consequences of these mutations are largely unknown. We have identified two amino acid changes (G21R and R75Q) that have an autosomal dominant pattern of hearing loss transmission, other changes inherited in an autosomal recessive fashion (L90P, P173R, M1V, R143W) and other changes of uncertain clinical consequences (M34T, R127H, V95A). Functional analysis by dye transfer experiments in HeLa cells stably transfected with the wild type or mutated GJB2 coding region, on some GJB2 mutations, such as M34T, R127H, or R184P showed that mutations M34T or R184P have a reduced functionality. In contrast, mutation R127H, which had been proposed as a dominant mutation, since it was detected in a subject with a dominantly inherited deafness, is in fact as functional as the wild type connexin, and thus, it is probably a polymorphism. This is supported by the identification of additional families and the absence of segregation with hearing impairment. We are also performing new functional studies for G21R, R75Q, V95A and A149T, also by dye transfer in HeLa cells. Acknowledgements: RR is supported by a BEFI grant (98/9207). The study has been supported by a grant from the Botin Foundation and the FISS (Spanish Ministry of Health).

**Mutational analysis of Peroxiredoxin IV (Prx-IV): exclusion of a positional candidate for multinodular goiter.** *E. Giardina*<sup>1</sup>, *F. Capon*<sup>1</sup>, *S. Sciacchitano*<sup>2</sup>, *R. Bruno*<sup>3</sup>, *V. Tassi*<sup>4</sup>, *V. Trischitta*<sup>4</sup>, *S. Filetti*<sup>5</sup>, *B. Dallapiccola*<sup>2,6</sup>, *G. Novelli*<sup>1</sup>.  
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Multinodular goiter (MNG) is a common disorder characterized by an enlargement of the thyroid, occurring as a compensatory response to hormonogenesis impairment. We recently assigned an MNG locus to chromosome Xp22 (MNG2, OMIM \*300273), in a region containing the peroxiredoxin IV (Prx-IV) gene. Prx-IV, previously known as AOE372, belongs to a family of antioxidant enzymes, regulating intracellular concentration of reactive oxygen species, including hydrogen peroxide. H<sub>2</sub>O<sub>2</sub> concentration is critical in thyroid tissue, where it is utilized for thyroxine synthesis. In this study, we screened Prx-IV as a positional candidate gene for the MNG2 locus. We sequenced the gene 7 coding exons in 2 patients and 2 unrelated controls. We identified two adjacent polymorphisms within intron 1, but failed to detect any mutation within the coding sequence. On the whole, our data exclude the involvement of Prx-IV in MNG pathogenesis. Work funded by the Italian Telethon (Grant E1031).

**FSHD Myoblasts Possess Reduced Resistance to Oxidative Stress.** *K.A. Barrett<sup>1,2</sup>, R. Tawil<sup>2</sup>, R.C. Griggs<sup>2</sup>, D.A. Figlewicz<sup>1,2</sup>.* 1) Dept Neurobiology & Anatomy, Univ Rochester, Rochester, NY; 2) Dept Neurology, Univ Rochester, Rochester, NY.

Facioscapulohumeral muscular dystrophy (FSHD), the third most common muscular dystrophy, is inherited in an autosomal dominant manner. A variable deletion in a repeat region (D4Z4) of chromosome 4q35 has been associated with the disorder, however, the pathogenesis of FSHD has yet to be established. Myoblasts from FSHD patients possess a necrotic appearing morphology, with a swollen cytoplasm and perinuclear vacuoles. Highly confluent cells appear to lack the organization of normal myoblasts, and FSHD cells fuse to form disorganized, swollen myotubes. Although necrotic appearing cells can be found in cultures of normal myoblasts, a larger percentage of FSHD cells possesses the phenotype ( $2.6 \pm 1.5\%$  for normals, and  $15.7 \pm 6.1\%$  for FSHD,  $p=0.046$ ,  $n=4$ .), controlling for cell density and replicative age. Preliminary observations indicate that this phenotype can be elicited in normal cells exposed to the superoxide anion generator, paraquat. Additionally, previous studies have demonstrated an increased susceptibility of FSHD myoblasts to paraquat relative to normal and disease control (other myopathies) cells. The cyclin dependent kinase (cdk) inhibitor, p21, appears to be upregulated in FSHD cells under normal growth conditions (22.2% of FSHD myoblast nuclei stain strongly positive for p21 compared to 14.2% of normal myoblast nuclei,  $p=0.004$ ,  $n=4$ .); a similar phenomenon in fibroblasts was associated with oxidative stress. Rescue from oxidative stress was attempted by exposing normal and FSHD myoblasts to the membrane permeable anti-oxidant, glutathione ethyl ester (GSH-OEt). Low concentrations of GSH-OEt (0.89mM) achieved full rescue of normal cells exposed to 20mM paraquat. In contrast, GSH-OEt was unable to rescue FSHD cells exposed to 10mM paraquat; the viability of FSHD myoblasts in test wells (containing paraquat plus GSH-OEt) was equivalent to that in wells containing paraquat alone. The current study demonstrates an enhanced irreversible vulnerability of FSHD myoblasts to oxidative stress, suggesting a biochemical marker for FSHD early in myocyte development.

**A KO mouse model for SURF-1 deficiency.** *A. Agostino, L. Valletta, V. Tiranti, P. Corona, M. Zeviani.* Molecular Neurogenetics, Neurological Institute, Milan, MI, Italy.

Loss-of-function mutations of SURF1 cause Leigh syndrome and isolated, generalized COX deficiency in humans. A constitutive KO mouse for SURF1 was generated by replacing exons 3-5 of the murine gene with a Neo cassette. Two independent heterozygous Surf1<sup>+/-</sup> ES cell clones were used to generate three 100% Surf1<sup>+/-</sup> germline chimaeras. Several Surf1<sup>+/-</sup> F1 heterozygous animals were mated together to produce F2 litters. We obtained only three at-term Surf1<sup>-/-</sup> pups out of approximately 150 F2 individuals, suggesting that the KO Surf1<sup>-/-</sup> genotype is embryolethal in most of the cases, at least in our experimental conditions. No Surf1 specific cross-reacting material was detected in fibroblasts from two Surf1<sup>-/-</sup> individuals, and from several organs of a third Surf1<sup>-/-</sup> individual sacrificed at one month after birth. In this animal, COX activity was 40% and 10% of the controls means in muscle and liver homogenates, respectively. The other respiratory chain complexes were normal. This result indicates a function for murine Surf1 specifically related to COX. Brain and muscle histology were both normal. Body growth rate appeared markedly delayed in all three Surf1<sup>-/-</sup> animals and body weight has remained stably reduced in the two KO animals that reached adulthood, compared to age-matched controls. At 5 months after birth, no overt neurological abnormalities have been detected in both. We are currently carrying out further studies on the phenotypic characterization of the KO <sup>-/-</sup> animals, the expression of the surf1 gene cluster in <sup>-/-</sup> and <sup>-/+</sup> individuals, and identification of the embryonal stage of <sup>-/-</sup> lethality .

**Human cationic trypsinogen gene: a new paradigm of disease-associated genes resulting in a “gain-of-function” condition.** *T. Montier, J.M. Chen, C. Férec.* INSERM-EMI 01 15, Etablissement Français du Sang-Bretagne, Université de Bretagne Occidentale, and Centre Hospitalier Universitaire, 46 rue Félix Le Dantec, 29275 Brest, France.

A first classification of mutations is into “loss-of-function” vs “gain-of-function” mutations. It is widely accepted that “gain-of-function” is likely when only a specific mutation in a gene produces a given pathology. However, the identification of multiple, pancreatitis-associated “gain-of-function” mutations in the human cationic trypsinogen gene (PRSS1) is modifying this classical perception.

To date, at least five missense mutations—A16V, D22G, K23R, N29I, and R122H—in the PRSS1 gene have been found to be associated with hereditary or/and sporadic pancreatitis, a much wider mutational spectrum than previously thought with respect to a “gain-of-function” condition. This surprising finding appears to lie in the complicated biogenesis of trypsin and the complex regulation of trypsin(ogen) activation/inactivation: A16V affects the signal peptide cleavage site, D22G and K23R affect the activation peptide cleavage site, R122H disrupts the important R122 autolysis site, and N29I enhances zymogen autoactivation or increases trypsin stability, all of which resulting in an enhanced trypsin activity within the pancreas. Clearly, “gain-of-function” of trypsin can occur through a variety of mutations that affect different amino acid residues with important biological significance. This will make PRSS1 mutations a unique example showing that the spectrum of “gain-of-function” mutations in a gene is not always restricted. Moreover, unlike some genes, where one or two mutations cause a “gain-of-function” condition but other mutations produce different syndromes, the PRSS1 gene may be only associated with a “gain-of-function” condition. This is because on one hand, trypsin appears to perform no function other than alimentary digestion. On the other hand, deficiency resulting from any “loss-of-function” mutations in PRSS1 will not produce a disease due to the existence of multiple functional trypsinogen genes.

**Type II collagen is abnormally processed in spondyloepiphyseal dysplasia tarda (SEDL) cartilage.** *G.E. Tiller<sup>1</sup>, C.E. Baird<sup>1</sup>, M.A. Weis<sup>2</sup>, D.R. Eyre<sup>2,3</sup>.* 1) Dept of Pediatrics, Vanderbilt Univ School of Medicine, Nashville, TN; 2) Dept of Orthopedics, Univ of Washington, Seattle, WA; 3) Dept of Biochemistry, Univ of Washington, Seattle, WA.

Spondyloepiphyseal dysplasia tarda (SEDL) is an X-linked skeletal dysplasia characterized by disproportionate short stature and early-onset osteoarthritis. The SEDL gene is located at Xp22.2, and encodes a 140 amino-acid protein provisionally named sedlin. In addition, a transcribed retropseudogene exists on chromosome 19. Mutational analysis of the SEDL gene in SEDL patients has revealed a recurrent splice mutation, IVS3+5G®A, which is predicted to eliminate the translational start site. In order to assess the effects of the mutation on gene expression, immunohistochemical analysis of cultured fibroblasts using an anti-sedlin antibody was performed. Control fibroblasts demonstrated a perinuclear staining pattern, suggestive of ER or Golgi localization, whereas SEDL fibroblasts demonstrated no staining above background. We have also performed biochemical analysis of articular cartilage from a 50-year old SEDL male with this mutation. Electrophoresis of type II collagen CNBr peptides revealed retarded migration of all major peptides, which was comparable to that seen in tissue from SED congenita patients with type II collagen gene (COL2A1) mutations, and more extensive than that seen in patients with idiopathic osteoarthritis. These results are consistent with post-translational overmodification of type II collagen  $\alpha$ -chains. These data suggest that the common IVS3+5G®A mutation in the SEDL gene is a null allele; the retropseudogene on chromosome 19 is transcribed but not translated; and type II collagen undergoes aberrant post-translational modification in SEDL chondrocytes. Our data support the hypotheses that sedlin serves a role in trafficking of extracellular matrix components within chondrocytes, and that overmodification of type II collagen may be responsible in part for the SEDL phenotype.

**Fibrillin-1 Abnormalities in Fibroblast Cultures From First Degree Relatives of Patients with Systemic Sclerosis.**

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Systemic sclerosis (SSc) is a multisystem disease characterized by visceral and cutaneous fibrosis, autoantibodies, and microvascular abnormalities. We have previously shown that fibroblast cultures from systemic sclerosis (SSc) patients assemble fibrillin-1 containing microfibrils that are more sensitive to degradation than microfibrils assembled by control cells. In addition, FBN1 polymorphisms (SNPs) have been found to be associated with SSc in two populations. We sought to determine if fibrillin-1 microfibril instability could be detected in fibroblast cultures from 1<sup>st</sup> degree relatives of SSc patients. Fibrillin-1 is synthesized as a proprotein and after secretion and proteolytic processing, it multimerizes into matrix structures termed microfibrils. Fibroblasts were explanted from the skin of unaffected 1<sup>st</sup> degree relatives (N=22), of SSc patients (N=12), and age matched controls (N=14). After the cells were metabolically labeled with [<sup>35</sup>S] cysteine for 3 hours, then incubated without label present. The media, cellular lysate, and ECM components were harvested at 24 and 96 hours. The amount of fibrillin-1 in the ECM was quantified on triplicate experimental sets after SDS-PAGE using a Molecular Dynamics Storm 860 imager and a ratio was established using an intracellular protein as a control. Statistical analysis was performed with the Mann-Whitney U test. Analysis of the cellular lysates and medium showed efficient secretion and proteolytic processing from fibrillin-1 in the fibroblast cultures of the 1<sup>st</sup> degree relatives. In contrast, assessment of the amount of fibrillin-1 in the ECM of fibroblasts from 1<sup>st</sup> degree relatives indicated that the amount of fibrillin-1 was significantly lower at 24 hours ( $p < 0.0001$ ), and at 96 hours ( $p = 0.0001$ ) as compared with controls, although a pattern of inheritance of this abnormality could not be determined. These studies indicate that decreased fibrillin-1 incorporation into the ECM is apparent in the cultures from unaffected 1<sup>st</sup> degree relatives of patients with SSc, which supports the hypothesis that the abnormalities in fibrillin-1-containing microfibrils in SSc have a genetic basis, but other genetic or environmental factors are required for the SSc phenotype.



**Identification of a Locus for Anophthalmia on Chromosome 3q, with Delineation of a 2.3 Megabase Candidate**

**Interval.** *A. Male*<sup>1</sup>, *A. Davies*<sup>1</sup>, *A. Bergbaum*<sup>1</sup>, *J. Keeling*<sup>3</sup>, *L. Grace*<sup>4</sup>, *D. Fitzpatrick*<sup>5</sup>, *C. Mackie Ogilvie*<sup>1</sup>, *J. Berg*<sup>2</sup>. 1) The Genetics Centre, Guy's Hospital, London; 2) Division of Medical and Molecular Genetics, GKT School of Medicine, London; 3) Department of Paediatric Pathology, Royal Hospital for Sick Children, Edinburgh; 4) Department of Clinical Genetics, Western General Hospital, Edinburgh; 5) MRC Human Genetics Unit, Western General Hospital, Edinburgh.

Anophthalmia occurs in 3-6 per 100,000 individuals, either in isolation or as a part of a dysmorphic syndrome. In most cases of anophthalmia no cause is identified; however, there are autosomal recessive and X-linked forms of the disease. We have identified two dysmorphic children with bilateral anophthalmia in association with a chromosomal deletion involving distal chromosome 3q. Both patients have bilateral anophthalmia in association with intra-uterine growth retardation, microcephaly, a broad forehead and triangular face. The first patient has a complex karyotype with a translocation between chromosomes 3 and 7 and a deletion of a small segment of chromosome 3 (3q26-q28). The second patient had additional features of partial agenesis of the corpus callosum, abnormal neuronal migration, midline cleft palate, a laryngeal cleft, 13 pairs of ribs and micropenis. He has an interstitial deletion involving a small region of distal chromosome 3q that is not yet fully defined. We have identified BACs known to map to distal 3q from the Ensembl database, and have used fluorescence in-situ hybridization to determine the minimum deleted region common to both patients. The minimum deleted interval identified to date is between clones RPC11-134F2 and RPC11-430L16, an interval of approximately 2.3 million base pairs. We hypothesise either that haploinsufficiency for a gene in this interval is sufficient to cause anophthalmia, or that the chromosomal deletion is unmasking a recessive gene for anophthalmia on chromosome 3q. Our candidate interval contains 12 known genes and 7 novel predicted transcripts, but excludes the HRY gene which was previously a strong candidate. Further characterisation of the candidate interval is in progress.

**Development of a cDNA microarray to study muscular dystrophy.** *T. Tsukahara*<sup>1</sup>, *H. Nagasawa*<sup>2</sup>, *K. Arahata*<sup>1, 3, 4</sup>. 1) Dept Neuromuscular Research, National Institute of Neuroscience, NCNP, Tokyo, Japan; 2) Ebara Co., Kanagawa, Japan; 3) CREST, JST, Tokyo, Japan; 4) Dr. Kiichi Arahata passed away on December 20, 2000.

Defects of plasma membrane or extracellular matrix associated proteins, dystrophin or laminin alpha 2 and so on, due to fragile sarcolemma and muscular dystrophy have been identified. However, there are some muscular dystrophy genes whose products are not associated with the plasma membrane. In particular, deficiencies in two nuclear membrane associated proteins, emerin and lamin A/C, result from Emery-Dreifuss muscular dystrophy, EDMD. Moreover, genes for some cytosolic enzymes, calpain 3 and myotonin protein kinase are also responsible genes for muscular dystrophy. However, the relationship between abnormalities in these genes and muscular dystrophy is unclear. Recently, the characterization of pathological features by a comprehensive examination of gene expression in the patients tissue has become possible. To clarify the gene expression profile and to help therapeutic studies, we developed a human muscle cDNA microarray.

To develop a low-background microarray, we constructed a highly nonredundant human singleton database for virtual cDNAs expressed in skeletal or cardiac muscle. The database was constructed from public sequence data, and then homologous sequences with rRNA and with mtDNA, and with repetitive sequences were excluded to increase the reliability of each probe. PCR primers were designed as amplification lengths of 450-550bp in the vicinity of 3 ends of each singleton. Each fragment of genes in the database was amplified with specific primers and muscle cDNA pools, cloned and confirmed by sequencing. 1536 clones were then amplified, purified and spotted on a CMT-GAPS coated slideglass to make microarrays.

To confirmed sensitivity and reproducibility of our microarray, RNAs were labeled and then analyzed by using tyramide signal amplification system and the ScanArray 5000. One microgram of total RNA was enough to analyze, and our microarray showed low background and good resolution. This microarray was considered to be a suitable device for gene expression analysis of the muscular disease with biopsied samples.

**Al-Aqeel Sewairi Syndrome, A new autosomal recessive disorder with multicentric osteolysis and arthritis with a novel mutation of matrix metalloproteinase 2 gene (MMP-2).** *W.M. Al sewairi<sup>1</sup>, A.I. Al-Aqeel<sup>1</sup>, R.J. Desnick<sup>2</sup>, J.A. Martigenetti<sup>2</sup>.* 1) Departement of Pediatrics, Riyadh Military Hospital , Riyadh, Sauda Arabia; 2) Departement of Human Genetics, Mount Siani School of Medicine, New York, USA.

We reported an autosomal recessive multicentric osteolysis in a Saudi Arabian family with distal arthropathy of the metacarpal, metatarsal and interphalangeal joints, which eventually progressed to the proximal joints and resulted in ankylosis and generalized osteopenia. In addition, they had large, painful to touch palmar and plantar pads and mild dysmorphic facial features including proptosis, a narrow nasal bridge, bulbous nose and micrognathia. Using a genome-wide search for homozygous-by-descent microsatellite markers from 6 members of this family, localized the disease gene to chromosome 16q12-21 with a LOD score of 4.59. Haplotype analysis with additional markers narrowed the critical region to 1.2 cM between markers D16S3032 and D16S3140 and identified the matrix metalloproteinase 2 (MMP-2 , gelatinase A, collagenase type IV, EC 3.4.24.24) gene as a disease candidate. All affected individuals were homoallelic for a nonsense mutation (TCA>TAA) in codon 244 of exon 5, predicting the replacement of a tyrosine residue by a stop codon in the first fibronectin type II domain (Y244X) leading to no MMP-2 enzyme activity in serum and/or fibroblast of affected individuals. In conclusion: The discovery that deficiency of this well characterized gelatinase/collagenase results in an inherited form of an osteolytic and arthritic disorder provides invaluable insights for the understanding of osteolysis and arthritis and the in vivo function of MMP-2.

**Truncating FgfR2 exon IIIc mutation in a familial form of Jackson-Weiss syndrome.** V. El Ghouzzi, E. Lajeunie, T. Hirtzig, J. Bonaventure, A. Munnich, M. Le Merrer. Human Genetics, Hopital Necker Enfants Malades, Paris, France.

Originally described in a large Amish family, Jackson-Weiss syndrome is a craniosynostosis syndrome with midface hypoplasia, hypertelorism, proptosis, foot abnormalities including broad great toes, partial syndactyly of the second and third toes and fusion of tarsal and metatarsal bones. All hitherto reported mutations are missense substitutions occurring within the third Ig-like loop of FgfR2 gene. Here, we report on severe bicoronal craniosynostosis with facial and foot abnormalities typical of the Jackson-Weiss syndrome in a mother and her son carrying a FgfR2 mutation in the alternatively spliced exon 9 which is specific for the IIIc isoform of the receptor. Sequencing this exon revealed heterozygosity of both patients for a two nucleotide deletion at codon 320 (958delAC) causing a frameshift and a premature termination codon at position 324. Surprisingly, semi-quantitative RT-PCR analysis of the patient fibroblasts revealed a 2-fold decreased expression of FgfR2 IIIc, suggesting that the mutation triggered mRNA instability of this isoform. As this mutation could cause ectopic expression of the FgfR2 IIIb isoform, expression of this isoform was investigated in patient fibroblasts but not found. Because no osteoblastic cells of the patients were available, it was not possible to test whether the mutation caused re-expression of FgfR2 IIIb in the calvaria. To our knowledge, this is the first report of a non sense mutation in FgfR2 associated with a craniosynostosis phenotype. Based on a recent FgfR2-IIIc +/- mouse model, we suggest that an heterozygous nonsense mutation in the FgfR2 IIIc isoform would cause a splicing switch resulting in a gain-of-function mutation.

**A Novel CBFA1 mutation in an Italian family CCD with skeletal myopathy.** *C. Casali<sup>1</sup>, F. Cricchi<sup>1</sup>, M. Spadaro<sup>1</sup>, L. Benedetti<sup>1</sup>, G. DiGiacinto<sup>1</sup>, G.A. Amabile<sup>1</sup>, A. Tessa<sup>2</sup>, F.M Santorelli<sup>1, 2</sup>.* 1) Inst Clinica Malattie Ner Men, La Sapienza Univ, Rome, Rome, Italy; 2) Molecular Medicine Unit, IRRCS Ospedale Pediatrico Bambino Ges, Rome, Italy.

Cleidocranial dysplasia (CCD) is a skeletal disorder characterized by large fontanelles, hypoplastic or aplastic clavicles, dental anomalies, and short stature. We studied a two-generation Italian family with a classic CCD phenotype in two patients, father and daughter. Interestingly they also presented clinical, EMG and morphologic signs of skeletal myopathy. We identified a novel CBFA1 mutation in exon 1 (c.398G>A) resulting in the replacement of tryptophan 116 by a stop codon (W116X). A diagnostic polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used for rapid detection of the novel mutation in the proband and his relatives using the endonuclease BfaI. The novel mutation is likely to be pathogenetic because of the following considerations: 1) the W116X segregated in affected family members; 2) it was not found in 150 control chromosomes; 3) it occurs in the highly conserved CBFA1 runt domain, resulting in premature translation termination. Our findings expand the list of CBFA1 allelic variants in CCD. The CBFA1 gene encodes a transcription factor required for osteoblast cell fate commitment. Identified mutations mostly reside within the DNA-binding runt homology domain. As in other cases, it is possible that the W116X mutation abolishes the DNA-binding ability of the transcription factor CBFA1, affecting protein function and leading to reduced function. Loss-of-function of one allele might result in delayed intramembranous and endochondral ossification since both these pathways have in common osteoblast cell fate commitment and differentiation as a final step in matrix ossification. If this relates to the muscular disorder in the propositus will require further studies.

**Genetic mapping and characterization of an autosomal recessive mouse mutation resulting in skeletal defects and male infertility.** *K.N. Bromfield<sup>1</sup>, I.J. Karolyi<sup>1</sup>, A.M. Wenglikowski<sup>1</sup>, D. Dolan<sup>2</sup>, G. Dootz<sup>2</sup>, A.A. Finnegan<sup>3</sup>, L.D. Siracusa<sup>3</sup>, S.A. Camper<sup>1</sup>.* 1) Human Genetics, University of Michigan Medical School, Ann Arbor, MI; 2) Otolaryngology, University of Michigan Medical School, Ann Arbor, MI; 3) Department of Microbiology and Immunology, Kimmel Cancer Center, Jefferson Medical College, Philadelphia, PA.

A spontaneous mutation resulting in growth insufficiency appeared in the progeny of a female DBA.B6-A<sup>hvy/a</sup> and her father, a DBA/2J male, at the N3 backcross generation. Further breeding demonstrated that the growth impairment was genetic and consistent with recessive inheritance. Mutant males are sterile, but mutant females are semi-fertile. There are no obvious neurological problems in the mutants but they exhibit hind leg clasping when held by the tail.

The growth impairment is detectable by weaning. Adult male and female mutants weigh approximately 75% of their normal littermates. Thyroid hormone levels are normal. Alcian blue and alizarin red staining reveals skeletal abnormalities in the mutant mice, including shortened skull, reduced vertebral bodies, flattened iliac crests, shortened fore and hind limbs and enlarged joints. The number of vertebrae and ribs is normal. These results suggest that the growth insufficiency is due to a developmental defect of bone or cartilage.

Mutant testes are only 25% the size of normal adult testes, although seminal vesicles are normal. Histology reveals small seminiferous tubules, no evidence of sperm maturation, and reductions in germ cells and Sertoli cells.

The karyotype of mutants is normal. To determine the genetic map location of the mutant locus, mutant females were crossed to *Mus castaneus* and 100 F1 intercross progeny were collected. Although growth insufficiency is less obvious among the intercross progeny, both it and male hypogonadism are fully penetrant. Pools of DNA from either mutant mice or their normal littermates were composed, and a genome scan with polymorphic markers revealed an association of the phenotype with distal chromosome 9. Analysis of positional candidate genes is in progress.

**CONTINUED GENETIC INVESTIGATION OF PATIENTS WITH PRESUMED DEFECTS IN THE GH-IGF-1 AXIS: GENOTYPE:PHENOTYPE CORRELATIONS.** *M.E. Ahearn, L. Gayol, E. Estrella, E. Volz, H. Basterrichea, M. Silverman, A. Davis, L. Baumbach.* Pediatrics, Univ Miami Med Sch, Miami, FL.

An increasing percentage of unexplained growth retardation are due to detectable genetic abnormalities, many of which occur in genes which encode proteins and/or factors in the GH-IGF-1 axis. The further understanding of the molecular basis of short stature syndromes will lead to design of better management and treatment modalities. We have completed molecular studies of the Pit-1 and Prop-1 loci in a cohort of short stature patients who have either isolated or multiple pituitary hormone deficiency and of the growth hormone receptor (GHR) gene in patients with suspected growth hormone insensitivity syndrome. Ten individuals representing six families were studied for Pit-1 mutations, 12 individuals in six families for Prop-1 mutations, and four probands in four families for GHR mutations. Possible mutations were evaluated through exon-specific PCR, SSCP and DNA sequencing of SSCP variants. Known mutations for each of these genes were included as positive controls. Evaluation of Pit-1 mutations has revealed a novel SSCP variant found in exon 5 in a consanguineous family with classical Pit-1 phenotype. We are awaiting DNA sequencing results, however, mutations in this exon have never been reported. The remaining five families, who did not display classical Pit-1 phenotypes, did not have detectable Pit-1 abnormalities. Screening for Prop-1 mutations focused initially on detection of a recurrent 2bp deletion mutation in exon 2, which will followed by complete SSCP analysis of the Prop-1 locus. To date, one family displaying a classical Prop-1 phenotype, carries the 2bp del mutation. The entire GHR locus was screened for possible mutations, and to date, no mutations were found in the patients studied, who did not have classical Laron syndrome. These combined results suggest that mutations in these loci will be most often found in patients with classical presentations, and that patients with variant phenotypes may well be due to mutations in-yet to be identified growth regulatory genes.

**Identification of novel mutations in the COL1A1 gene.** *V. Kucinskas, E. Benusiene, D. Kasperaviciute, R. Slibinskas.* Human Genetics Ctr, Vilnius Univ, Vilnius, Lithuania.

Osteogenesis imperfecta (OI) is a generalized disorder of connective tissue characterized by an increased fragility of bones and also manifested in other tissues containing collagen type I, by blue sclera, hearing loss, dentinogenesis imperfecta, hyperextensible joints, hernias and easy bruising. OI is dominantly inherited and results from mutations in one of the two genes (COL1A1 and COL1A2) for the type I procollagen. Recognising the molecular background of OI is helpful in proper diagnosis and, as a result, leads to an improvement of genetic counselling for the families at risk.

The aim of this study was to determine mutations which cause osteogenesis imperfecta (OI) in Lithuanian patients to better postnatal and prenatal diagnosis.

Thirteen familial and eight sporadic dominant OI cases (out of 110 recorded in the Lithuanian OI database) were available for molecular genetic testing. The segregation of OI-linked COL1A1 and COL1A2 loci was analysed using RFLP within or close to the genes. Comparison of phenotypic features with the concordant collagen locus showed that in three pedigrees Sillence OI type I segregated with the COL1A1 locus, while in two pedigrees Sillence OI type I and OI type IV segregated with the COL1A2 locus. In eight remaining pedigrees the data available were insufficient for the identification of a definite OI-linked COL1A locus. DNA heteroduplex analysis based screening for mutations in 30 exons of the COL1A1 gene was performed in probands from 11 OI families and 8 sporadic OI cases. The results showed the presence of nucleotide sequence changes segregating with the OI phenotype in seven probands. Using direct DNA sequencing seven mutations were identified. Out of them, six mutations appeared to be novel: E500X, c.2046-2047insCTCTCTAG, c.1668delT, c.1667-1668insC, IVS19+1G>A, IVS20-2A>G (nucleotides are numbered from the first base of the start codon).



**Cleidocranial dysplasia phenotype and correlations with CBFA1/RUNX mutations.** *K.L. McBride, D. Napierala, Y. Chen, G. Zhou, B. Lee.* Human and Molecular Genetics, Baylor College of Medicine, Houston, TX.

Cleidocranial dysplasia (CCD) is a dominantly inherited skeletal dysplasia with high penetrance and variable expressivity, characterized by delayed endochondral and intramembranous ossification. We and other groups previously reported that mutations in the osteoblast-specific transcription factor CBFA1 cause CCD. The CBFA1 protein consists of an N-terminal stretch of polyglutamine/polyalanine repeats, a runt domain homologous with the *Drosophila* runt gene, and a proline/serine/threonine rich activation domain. The majority of mutations are missense and affect the runt domain, abolishing DNA binding to a target OSE2 sequence. These and other mutations which result in premature termination of the protein in the runt domain produce the classic CCD phenotype by abolishing transactivation by the mutant protein and hence haploinsufficiency of the normal gene.

The objective of the current study was to describe further mutations of the CBFA1 gene associated with CCD and correlate with the respective phenotype. We performed PCR amplification of genomic DNA for CBFA1 gene and sequenced all of its 8 exons. We analyzed for previously undescribed mutations in 27 unrelated patients.

A total of 10 mutations in 10 individuals were identified. There were 3 missense mutations in the runt domain, R190P, R193L, and Q209H, and two frameshifts, 542delG and 867insC. There was one deletion (243del18) in the polyQ/A. Two nonsense mutations (Q284X, Q292X) and two frameshift mutations (961delG, 1224insC) occurred in the PST domain. No mutation types recurred in different families. The frameshift mutation 542delG occurred in a patient with CCD and multiple fractures. Cohort patients had clinical expressions ranging from isolated dental anomalies to severe CCD with fractures. The location of the mutations also correlates well with alteration of multiple interacting domains.

In conclusion, our data demonstrate that variable loss of CBFA1 function may be associated with a spectrum of classic CCD, CCD with osteopenia and fractures, and isolated dental anomalies.

**Identification of genetic loci that modify tooth and bone phenotypes.** *T.C. Hart<sup>1,3</sup>, M.L. Marazita<sup>2,3</sup>, Y. Zhang<sup>1</sup>, M.C. Gorry<sup>1</sup>, M.E. Cooper<sup>2</sup>, J.J. Marks<sup>1</sup>, J.T. Wright<sup>4</sup>.* 1) Dept. Oral Med/Pathology, Univ Pittsburgh Sch Dental Med, Pittsburgh, PA; 2) Dept. Oral & Max Surg, Univ Pittsburgh SDM, Pittsburgh, PA; 3) Dept. Human Gen, Univ Pittsburgh Grad Sch Public Health, Pittsburgh, PA; 4) Dept. Pediatric Dentistry, Sch Dentistry, Univ North Carolina, Chapel Hill, NC.

Tricho dento osseous syndrome (TDO) is an autosomal dominant condition that results from a 4 bp deletion mutation of the distal-less 3 gene (DLX3). We have identified 7 extended families segregating TDO. All 52 TDO affecteds share a common haplotype surrounding the same DLX3 mutation, suggesting it is inherited from a common ancestor. TDO is characterized by variable hair, teeth and bone expression. Thickness of the cranial bones measured radiographically at glabella (mean TDO affected = 9.78 mm; unaffected 7.5 mm) and nuchal crest (mean TDO= 17.3 mm; unaffected 13.3 mm) are significantly increased in TDO affecteds ( $p=0.02$  and  $0.01$  respectively). Tooth size (mean width TDO =11.5 mm, unaffected=13.5 mm) and enamel thickness (mean TDO = 0.42 mm, unaffected 1.5 mm) are significantly reduced in affecteds ( $p=0.0001$ ) compared with unaffected individuals. As TDO affected individuals share a common DLX3 mutation, we hypothesized that the clinical variability associated with tooth and bone traits is due to modifying genes. Segregation analyses of tooth length (TL), width (TW), bone nuchal crest width (NCW), & glabella width (GW) phenotypes are consistent with genetic transmission. To identify modifying genes for tooth and bone findings, we performed linkage studies. Using a candidate gene approach we identified support for genetic modifiers. The transcription factor DLX5 was significantly linked to TL ( $p<0.001$ ), TW ( $p=0.01$ ), GW ( $p=0.007$ ), & NCW ( $p=0.04$ ). Osteocalcin, was linked to TL ( $p<0.001$ ) and TW ( $p<0.001$ ), but not to bone measures. We tested the utility of a high density genome wide scan to detect linkage relationships for modifying genes. Linkage was detected for genetic loci  $> 2cM$  from the DLX5 locus with TL ( $p <0.001$ ), TW ( $p <0.001$ ) and NCW ( $p= 0.001$ ). These data suggest that linkage studies of families segregating TDO provide a unique human model to identify genetic modifiers of tooth and bone phenotypes. NIDCR DE10804.

**Camurati-Engelmann disease: New mutations in the latency-associated peptide of the transforming growth factor b-1 gene.** *S.R. Mumm<sup>1,2</sup>, S. Obrecht<sup>1</sup>, M.N. Podgornik<sup>2</sup>, M.P. Whyte<sup>1,2</sup>*. 1) Division of Bone & Mineral Diseases, Washington University School of Medicine and Barnes-Jewish Hospital Research Institute, St Louis, MO; 2) Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children, St. Louis, MO.

Camurati-Engelmann Disease (CED), also called progressive diaphyseal dysplasia, is an autosomal dominant disorder characterized by painful spreading hyperostosis of the diaphyses of major long bones beginning in childhood. Mutations in the latency-associated peptide (LAP)/transforming growth factor b-1 (TGFb-1) gene have been implicated as the cause of CED in reports of several Japanese and European patients. The LAP and TGFb-1 are co-expressed from the same gene as a single peptide, which is subsequently cleaved into separate LAP and TGFb-1 proteins. A dimerized LAP remains noncovalently bound to a dimerized secreted TGFb-1, presumably keeping the latter inactive. We have sequenced the coding region of the LAP/TGFb-1 gene in 8 unrelated CED patients (3 sporadic, 5 familial), identifying mutations in all but one sporadic case diagnosed elsewhere. In 4 of these probands, previously reported mutations were documented in exon 1 (9 bp insertion causing a 3 Leu insertion in a poly-Leu region) and in exon 4 (Arg218His, Arg218Cys). In the remaining 3 cases, new mutations were identified. The novel mutations include a dinucleotide change in exon 4 (Cys223Ser) at a critical residue involved in disulfide bonding and tertiary structure of the LAP; a previously reported single nucleotide mutation results in the same amino acid change (Cys223Ser). The second was a C to T transition in exon 2 (C463T) causing an arginine to cysteine change (Arg156Cys); one may speculate that this newly created Cys residue could also interfere with disulfide bonding and tertiary structure. The new mutations were not detected in 168 LAP/TGFb-1 alleles from unaffected individuals, showing the changes are mutations and not polymorphisms. Hence, all 7 of the mutations identified to date causing CED are located in the LAP; none are found within TGFb-1. This finding suggests the underlying defect in CED is control, at the protein level, of an intact TGFb-1.

**Targeted disruption of *Cacp* in the mouse resembles human Camptodactyly-Arthropathy-Coxa vara-Pericarditis syndrome (CACP) and provides insight into this disease's pathogenesis.** *J. Marcelino*<sup>1</sup>, *Y. Gong*<sup>1</sup>, *M. Baker*<sup>2</sup>, *M. Warman*<sup>1</sup>, *J. Carpten*<sup>2</sup>. 1) Dept Genetics, Case Western Reserve Univ, Cleveland, OH; 2) National Human Genome Research Institute, NIH, Bethesda, MD.

We have previously reported mutations in *CACP*, which encodes a large, multi-domain, secreted glycoprotein, as the cause of the autosomal recessive disorder Camptodactyly-Arthropathy-Coxa vara-Pericarditis syndrome (CACP). Patients with CACP have congenital or childhood-onset camptodactyly and develop a childhood-onset arthropathy characterized by a non-inflammatory synovial cell hyperplasia. To understand how mutations in *CACP* lead to joint failure, we targeted the *Cacp* gene in mice. Homozygous knock-out mice are viable and fertile. We performed histological analyses on the knee joints of wild-type, heterozygous, and homozygous mice at 15, 30, and 60 days of life. While the wild-type and heterozygous joints appeared normal, the homozygous joints revealed hyperplasia of the synovium, similar to that which has been described in biopsies from CACP patients. Homozygous knock-out mice also had abnormalities of their articular cartilage. The superficial zone of the cartilage appeared thickened and depleted of chondrocytes, and the articular surface had accumulated a layer of eosinophilic-staining material. Although signs of camptodactyly or arthropathy were not apparent in the homozygous knock out mice at 60 days of life, several animals developed swollen ankles and camptodactyly of their hind paws by 6 months of age. We conclude that the *Cacp* knock-out mice have features which resemble human CACP. The cartilage pathology we observed in these mice may also be present in CACP patients, but not appreciated because patients have not had articular cartilage biopsies. This animal model for CACP should permit us to explore potential roles for the CACP in joint homeostasis, such as the lubrication of articulating surfaces and the regulation of intimal cell growth.

**Identification of the gene resulting in the high bone mass trait linked to 11q13.** *R.D. Little<sup>1</sup>, J. Carulli<sup>1</sup>, R. Del Mastro<sup>1</sup>, J. Dupuis<sup>1</sup>, M. Osborne<sup>1</sup>, C. Root<sup>1</sup>, S.P. Manning<sup>1</sup>, P. Swain<sup>1</sup>, S-C. Zhao<sup>1</sup>, B. Eustace<sup>1</sup>, M.M. Lappe<sup>1</sup>, L. Spitzer<sup>1</sup>, S. Zweier<sup>1</sup>, K. Braunschweiger<sup>1</sup>, M.G. FitzGerald<sup>1</sup>, K.M. Allen<sup>1</sup>, S. Recker<sup>2</sup>, P. Van Eerdewegh<sup>1</sup>, R.R. Recker<sup>2</sup>, M. Johnson<sup>2</sup>.* 1) Human Genetics, Genome Therapeutics Corp, Waltham, MA 02453; 2) Osteoporosis Research Centre, Creighton University, Omaha, NE 68131.

Osteoporosis is a complex disease that affects over 10 million people in the U.S. and results in 1.5 million fractures every year. In addition, the high prevalence of osteopenia (low bone mass) in the general population places a large number of people at risk for developing the disease. In an effort to identify genetic factors influencing bone density, we characterized a family with individuals that possess exceptionally dense bones but are otherwise phenotypically normal. This autosomal dominant high bone mass trait (HBM) was originally localized to a 30 cM region on chromosome 11q12-13 by linkage analysis. In order to narrow the genetic interval we extended the pedigree and typed additional microsatellite markers. Linkage analysis resulted in a peak LOD score of 10.1 and a refinement of the interval to less than 3 cM. In order to identify candidate HBM genes, a physical map spanning the critical interval was developed and genomic sequencing of 15 BACs was performed. Comparative DNA sequencing was then used to identify polymorphisms in the HBM candidate genes. From our survey of 231 exons in 17 genes, a single polymorphism was identified that was only present in affected members of the HBM family and not in an ethnic diversity panel of ~1000 individuals. Inhibitors of bone resorption are the most common therapies for osteoporosis. However, the extent of bone loss may far exceed the amount that can be restored by these treatments, making stimulation of bone formation by specific osteogenic agents a potentially valuable adjunct therapy. Our findings suggest that the HBM mutation confers a unique osteogenic activity in bone remodeling, and that the HBM protein represents a novel therapeutic target for the treatment of osteoporosis.

**Physical Mapping of CHH Region and Mutation Analyses in RMRP Gene Among Amish and Non Amish**

**Patients.** *P.K. Jain*<sup>1</sup>, *S. Chandrashekarappa*<sup>2</sup>, *I. Makalowska*<sup>3</sup>, *C.A. Francomano*<sup>1</sup>. 1) Laboratory of Genetics, NIA, Baltimore, MD; 2) GMBB, NHGRI, NIH, Bethesda, MD; 3) GTB, NHGRI, NIH, Bethesda, MD.

Cartilage Hair Hypoplasia is an autosomal recessive disorder prevalent among the Old Order Amish in the United States and the Finish population, although sporadic cases exist in other populations as well. The CHH phenotype is characterized by short stature; sparse, fine hair and other associated features including deficient erythropoiesis, abnormal cellular immunity, aganglionic megacolon and increased risk of certain malignancies. The CHH locus was previously localized to approximately 1.5cM between D9S163 and D9S 1791. In the Amish population, we narrowed the critical region to less than a megabase between the markers D9S163 and D9S1804. We identified seven RPCI-11 library clones ( 182-N-22, 201-P-13, 395-N-21, 156-G-4, 331-F-9, 112-J-3 and 327-I-3) and one Research Genetics library clone 167-M-7 which constitute the contig of the critical region. We mapped several ESTs/Genes and markers within this region. One mapped EST (Accession No. N36356, Lod Score 8.30, distance 33cRS from SHGC33250 on Stanford G3 panel) within the region from Melanocyte library had more than 96% identity with the RMRP (RNA component of Mitochondrial RNA-Processing Endoribonuclease) gene. This gene is within the RPCI-11 library BAC clone 331-F-9 and Research Genetics BAC clones 98-A-4 and 468-J-10. The RMRP gene is encoded in the nucleus and transported to mitochondria and plays a role in RNA primer metabolism in mitochondrial DNA replication. Recently, mutations in this gene have been found among CHH patients (Maaret Ridanpaa et al; cell 104,195-203, 2001). We have screened the RMRP gene in our 15 patients from Amish population and 13 non-Amish patients and found that the mutation 70 A-->G is the more common mutation among our populations representing more than 75% alleles. Thus we conclude that in clinical practice this mutation should be screened first among CHH patients, but before making this, as a regular practice larger number of patients need to be studied.

**Missense Mutations in COL2A1 Which Alter Amino Acids Other Than Triple Helical Glycines in Type II**

**Collagen.** A.J. Richards<sup>1</sup>, J. Morgan<sup>2</sup>, H. Hughes<sup>2</sup>, C. Tysoe<sup>2</sup>, F.M. Pope<sup>2</sup>, A. Bird<sup>3</sup>, M.P. Snead<sup>4</sup>. 1) Pathology Department, Cambridge University, Cambridge, UK; 2) University Hospital of Wales, UK; 3) Moorfields Eye Hospital, London, UK; 4) Addenbrooke's NHS Trust, Cambridge, UK.

We have recently described a type II collagen R365C mutation in two sporadic cases of Stickler syndrome<sup>1</sup> (arthropathopathy). We have now detected a third instance of this mutation in a small family. We compare and contrast the resulting phenotypes to a large family, with an unclassified chondrodysplasia<sup>2</sup>, where a novel mutation (G1105D) is sited in a conserved region of the type II collagen C-propeptide. Unlike these first two cases where premature arthropathy is a feature, a COL2A1 L467F mutation<sup>1</sup> produces a predominantly ocular phenotype.

All affected individuals with the R365C mutation had the congenital membranous vitreous anomaly. Six individuals had suffered retinal detachment. The oro-facial phenotype showed moderate to mild midfacial and nasal hypoplasia. All had mild to moderate sensorineural hearing loss. Joint laxity was largely absent but degenerative arthropathy was severe in the 3 patients over 40yrs of age.

In a large family with a G1105D mutation, affected individuals exhibited a loose liquescent gel without the vitreous anomaly seen in the R365C patients. Some affected individuals showed early degenerative changes in the hips and patellae. Around the time of puberty, brachydactyly, due to abnormal phalangeal epiphyseal development became evident, and was a common feature in this family.

The family with a L467F change all had an "afibrillar" vitreous<sup>1</sup>. Retinal detachment and blindness was common resulting in a severe ocular phenotype. Referred with a diagnosis of Stickler syndrome, the hearing loss, arthropathy and midfacial hypoplasia were uniformly mild or absent in all affected individuals so that dominant rhegmatogenous retinal detachment (DRRD) might be a more appropriate description.

1 Richards AJ *et al* Am. J. Hum. Genet. 67:1083-94 (2000).

2 Hughes H *et al* 9<sup>th</sup> Manchester Birth Defects Conference (2000).

**Molecular diagnosis of SHOX abnormalities in Leri-Weill dyschondrosteosis.** *A.R. Zinn<sup>1</sup>, F. Wei<sup>1</sup>, H. Chen<sup>1</sup>, L. Zhang<sup>1</sup>, P. Marttila<sup>2</sup>, J.L. Ross<sup>3</sup>.* 1) McDermott Ctr Human Growth/Dev, Univ Texas SW Medical Sch, Dallas, TX; 2) Esoterix Endocrinology, Calabasas Hills, CA; 3) Dept. of Pediatrics, Thomas Jefferson University, Philadelphia, PA.

**INTRODUCTION:** Leri-Weill dyschondrosteosis (LWD, MIM 127300) is a dominant skeletal dysplasia characterized by short stature, mesomelia, and Madelung deformity. Mutations or deletions resulting in haploinsufficiency of the Xp/Yp pseudoautosomal gene SHOX have been identified in 60-80% of LWD cases in previous studies. We sought to determine the frequency and nature of SHOX abnormalities in a large number of U.S. LWD subjects. **METHODS:** We collected 29 unrelated families with one or more members having LWD (59 affecteds total) and tested at least one affected individual from each family for SHOX abnormalities. We used FISH to detect SHOX deletions and denaturing HPLC and/or direct sequencing to detect point mutations in the SHOX coding region. **RESULTS:** SHOX deletions were present in 23 families (79%) with a total of 44 affecteds (75%). Point mutations that cosegregated with the LWD phenotype were present in 5 families (17%) with a total of 13 affected (22%). These included frameshift, nonsense, and missense mutations that are all predicted to alter critical regions of the SHOX protein. We did not detect a SHOX deletion or point mutation in one family with two affected sisters. However, analysis of multiple intragenic single-nucleotide polymorphisms failed to demonstrate heterozygosity, suggesting that these women are hemizygous for a SHOX deletion too small to detect by FISH. We are attempting to confirm this deletion by Southern blotting and by segregating SHOX alleles using somatic cell hybrids. **CONCLUSIONS:** SHOX abnormalities are present in 96-100% of LWD patients. Deletions are more common than point mutations. SHOX abnormalities can be identified in nearly all LWD patients using a combination of molecular, cytogenetic, and genetic methods. We found no evidence for locus heterogeneity in LWD.



**Langer mesomelic dysplasia: clinical features and SHOX mutational analysis.** *J.L. Ross<sup>1</sup>, C. Scott<sup>2</sup>, P. Marttila<sup>3</sup>, F. Wei<sup>4</sup>, A.R. Zinn<sup>4</sup>.* 1) Dept Pediatrics, Thomas Jefferson Univ, Philadelphia, PA; 2) A.I. DuPont Children's Hospital, Dept Pediatrics, Jefferson Univ, Philadelphia, PA; 3) Esoterix Endocrinology, Calabasas Hills, CA; 4) McDermott Ctr Human Growth/Dev, Univ of Texas SW Medical Sch, Dallas, TX.

**INTRODUCTION:** Langer type mesomelic dysplasia (LMD, MIM 249700) is thought to be due to homozygous SHOX loss of function mutations. Radiologic findings include severely shortened long bones of the limbs, Madelung deformity, varus deformity of the humeral head, angulation of the radial shaft and distortion of the carpals, short femoral neck, and hypoplastic or absent proximal half of fibula. Homozygous SHOX deficiency was recently reported in a young Langer boy. We present the results of a detailed evaluation of four adults with LMD. **METHODS:** Each subject had careful auxological measurements as well as molecular examination of SHOX. **RESULTS:** Four LMD adults (including one sib pair) were evaluated (2 males, 2 females, ages 33-85 years). Mean height z-scores of the males and females were similar:  $-6.6 \pm 0.5$  vs.  $-6.1 \pm 0.9$ . All subjects had severe mesomelia: mean arm span z-score =  $-8.5 \pm 0.4$ , mean lower leg length z-score =  $-7.1 \pm 2.7$ , evidence of hypoplastic fibulae, and all had Madelung wrist deformity. Hands and feet were relatively normal-sized, and facies were normal without dysmorphic features. Deletion of one SHOX allele in the sibs with LMD was detected by FISH. A missense mutation in one SHOX allele was detected by sequencing in the other unrelated LMD woman. The remaining SHOX alleles are being analyzed. **CONCLUSION:** LMD was previously associated with complete deficiency of SHOX. Our LMD patients also show SHOX abnormalities, although we have not yet proved homozygosity. The resulting mesomelia is severe and is associated with severe short stature and Madelung deformity. The target bones of SHOX action appear to be specific with relative sparing of the hands, feet, and facies. Interestingly, the fibula is markedly abnormal in LMD but spared with SHOX haploinsufficiency. Males and females are similarly affected. Future studies are aimed at elucidating the mechanism of LMD.

**Molecular and protein studies of spondyloepiphyseal dysplasia tarda (SEDТ). X. Zhang<sup>1</sup>, S. Obrecht<sup>1</sup>, M.P. Whyte<sup>1,2</sup>, S. Mumm<sup>1,2</sup>.** 1) Division of Bone and Mineral Diseases, Washington University School of Medicine and Barnes-Jewish Hospital Research Institute, St. Louis, MO; 2) Center for Metabolic Bone Disease and Molecular Research, Shriners Hospital for Children, St. Louis, MO.

Spondyloepiphyseal dysplasia tarda (SEDТ) is an X-linked disorder that presents during childhood with back pain and short stature in boys, caused by vertebral malformation and degenerative changes involving the spine and major joints. The underlying defect is mutation in a gene designated *sedlin*. We have previously described two large SEDТ kindreds with either 2 or 5 base pair deletions in the *sedlin* gene. We now report on a unique splice site mutation in an African-American male with SEDТ. This mutation changes the splice acceptor site at exon 6 from the canonical AG to AC, which likely would prevent mRNA splicing at this site. This is the 3'-most mutation reported for SEDТ, but causes the classical symptoms of the disease.

To begin to understand the cellular function of *sedlin*, we have initiated expression studies and have made a *sedlin* antibody. The *sedlin* cDNA was expressed from an in-vitro translation system as an approximately 14 kD protein; this is consistent with the size predicted from the open reading frame for the protein without post-translational modifications. The *sedlin* antibody was generated with a *sedlin*-specific peptide in rabbits, and was shown to recognize the in-vitro translated protein by Western blot analysis. Endogenously-expressed *sedlin* was also identified as a 14 kD protein in SAOS cells, by Western blot. This results supports the notion that *sedlin* protein does not undergo significant post-translational modification. We have previously shown by Northern blot analysis that *sedlin* mRNA is expressed ubiquitously in a variety of tissues that we examined. In our preliminary Western blot experiments, however, the *sedlin* protein doesn't appear to be ubiquitously expressed. This may help explain why disease-causing mutations in the *sedlin* gene only appear to affect endochondral bone formation.

**A missense mutation in a calcium-channel gene causes SCA in a four generation family also with hemiplegic migraine.** *I. Alonso<sup>1,2</sup>, A. Tuna<sup>3</sup>, J. Coelho<sup>1</sup>, J. Barros<sup>3</sup>, J. Sequeiros<sup>1,2</sup>, I. Silveira<sup>1,2</sup>, P. Coutinho<sup>4</sup>.* 1) Unigene, IBMC - Univ Porto, Porto, Portugal; 2) Lab. de Genética Médica, ICBAS - Univ Porto, Porto, Portugal; 3) Hospital de St. António, Porto, Portugal; 4) Hospital de So Sebastião, Feira, Portugal.

Familial hemiplegic migraine (FHM) is a neurological disorder, characterized by episodes of migraine with hemiparesis or other neurological focal deficits, inherited in an autosomal dominant form. FHM can also be associated with fever, confusion, coma or ataxia. Seven different missense mutations in a gene that encodes a subunit of a neuronal calcium channel cause FHM. Mutations disrupting this gene are also involved in episodic ataxia type 2 (EA2), whereas expansion of a CAG repeat causes spinocerebellar ataxia type 6 (SCA6). We ascertained 29 individuals from a four-generation family, comprising 14 patients with multiple and different phenotypes of progressive cerebellar ataxia, hemiplegic migraine, focal neurological deficits and coma triggered by minor head trauma, dominantly inherited. We have (1) assessed CAG repeat size at the SCA6 locus; (2) performed linkage analysis with chromosome 19p markers D19S1150, D19S840 and D19S226 and (3) screened our family for mutations in the CACNA1A gene. Polymorphism detection was performed by PCR amplification, single strand conformational polymorphism (SSCP) analysis and sequencing. Expansions of the CAG repeat were not present in any of these patients. Linkage analysis was performed and showed positive lod scores to chromosome 19p markers. SSCP and sequencing detected a G-to-A substitution in exon 13 of the CACNA1A gene, resulting in an arginine to glutamine change at codon 583, previously described. This mutation is present in all 12 living patients and also in one at-risk individual. In conclusion, the same missense mutation in the CACNA1A gene gives rise to varied clinical syndromes of permanent ataxia, hemiplegic migraine and episodes of focal deficits or coma triggered by minor head trauma.

**Mutation spectrum and genotype-phenotype correlation of MECP2 in 100 Japanese patients with Rett syndrome.**

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Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder characterized by cognitive and adapted regression with autistic behavior, stereotypic hand movements, epilepsy and ataxia. Over 120 different mutations in the methyl-CpG binding protein 2 (MECP2) have been reported in patients with RTT, but the genotype-phenotype correlation has not been established. We have studied MECP2 mutations in 136 patients with RTT and 36 different mutations were detected in 100 patients. Common mutations were four missense mutations (R133C, P152R, T158M and R306C) detected in 34 cases and four nonsense mutations (R168X, R255X, R270X and R294X) detected in 37 cases. Comparing phenotypes in patients with the common MECP2 mutations, three mutations (R133C, R306C and R294X) were more frequently detected in patients with atypical RTT phenotypes. In particular, patients with R133C had the mildest clinical features; 4 in 7 patients with R133C had preserved speech variant type of RTT. Most patients with other common mutations (T158M, R168X, R255X and R270X) and frameshift mutations with deletion/insertion in C-terminal regions had typical form of RTT. A patient with a truncating mutation in 5' region of functional domains had congenital form of RTT. Influence of skewed X-inactivation detected in MECP2 region could be neglected in most patients with RTT. These results suggest that there is genotype-phenotype correlation of MECP2 in patients with RTT, although large scale study of adults patients with RTT needs to determine more precise influence of MECP2 mutations in clinical natural history and phenotypes.

**Genetic heterogeneity and functional candidate gene analysis in Joubert syndrome.** *C.L. Bennett<sup>1</sup>, I.P. Blair<sup>2</sup>, R.R. Gibson<sup>1</sup>, P.F. Chance<sup>1</sup>.* 1) Dept Pediatrics, Univ Washington, Seattle, WA; 2) Garvan Institute of Medical Research, Sydney, Australia.

Joubert syndrome (JS) is a rare autosomal recessive malformation syndrome involving agenesis or dysgenesis of the cerebellar vermis with accompanying brainstem malformations. The disease is also characterized by hypotonia, developmental delay, abnormal respiratory patterns, and abnormal eye movements. The biochemical and molecular mechanisms underlying the JS are unknown. JS is clinically heterogeneous and linkage analyses indicate that there is genetic heterogeneity. Recently, homozygosity mapping identified a Joubert locus on chromosome 9q34 in a consanguineous family of Omani origin and excluded the same locus in a second Iranian pedigree (Saar et al. *AJHG* 1999). We undertook haplotype analyses in 26 Joubert pedigrees including three consanguineous families, using markers that define the locus on chromosome 9q34. We found no evidence of homozygosity for markers in the 9q34 region in these JS pedigrees suggesting that other loci are yet to be identified. A second locus for JS was more recently suggested following the identification of a chromosome 17p11.2 deletion in a patient with features of the Smith-Magenis syndrome and JS (Natacci et al. *AJMG* 2000). The possibility that the JS-like phenotype seen in this individual was due to the unmasking of a recessive JS allele on the non-deleted chromosome was proposed. We undertook haplotype analyses in the 26 Joubert families mentioned above with markers spanning the 17p11.2 deleted region. We found no evidence for homozygosity in this interval in these pedigrees. We suggest that the majority of JS patients do not have a mutant gene in 9q34 or 17p11.2. More directly, we have been able to exclude EN1, EN2 and FGF8 as functional candidate genes by mutation analysis and *BARHL1* by fine mapping exclusion in our cohort of Joubert families. Other strong candidates await investigation including *GBX2*, *MATH1*, *PAX2*, and *PAX5*.

**Additional co-author: Paola Rimessi.**

Program Nr: 2432

**MECP2 TRANSCRIPTION ANALYSIS AND X-INACTIVATION PATTERN IN A RETT GIRL BRAIN TISSUE.** *F. Gualandi<sup>1</sup>, E. Manzati<sup>1</sup>, S. Bigoni<sup>1</sup>, M. Vacca<sup>2</sup>, M. D'Esposito<sup>2</sup>, G. Hajek<sup>3</sup>, M. Zappella<sup>3</sup>, E. Calzolari<sup>1</sup>, A. Ferlini<sup>1</sup>.* 1) Dept Exp Diagn Medicine, Medical Genetics Section, Ferrara, Italy; 2) IIGB-CNR, Napoli, Italy; 3) Neuropsichiatria Infantile, University of Siena, Italy.

A variety of different MeCP2 mutations have been identified in Rett syndrome (RTT) patients. Furthermore, many mutations, some of these exclusively, have been identified in a relevant number of FRAXA negative, X-linked aspecific mental retardation (X-AMR). Nevertheless, the true pathogenetic mechanism responsible for RTT phenotype(s) as well as the reason why some mutations are associated with X-AMR, remain still obscure. A clear genotype/phenotype correlation has not been established and the non-random X- inactivation remains the more convincing mechanism possibly underlying the diversity in the clinical features. However, the X-chromosome methylation pattern has only been evaluated in lymphocytes, raising the question as to how these cells can be representative of the brain scenario. In order to further examine the role of X-inactivation in RTT we performed transcription as well as X-inactivation HUMARA in a post-mortem sample (temporal cortex) from a RTT girl. Mutation detection in this patient revealed the presence of a R106W missense mutation. Gene-specific semiquantitative RT-PCR of MeCP2 RNA showed the normal transcript in RS brain cortex in amounts comparable to that in two control brains (a male temporal cortex and a female frontal cortex). However, direct sequencing of this transcript in RTT brain revealed only the mutated allele. X-inactivation studies on brain-derived DNA showed a clear skewed X inactivation (98 vs 2). These findings represent the first report on X-inactivation studies in RTT brain and show the occurrence of an extremely skewed inactivation at least in the tissue sample analysed. This evidence is not supporting the haploinsufficiency hypothesis and might be compatible with a loss of function pathogenetic mechanism. Acknowledgement The Italian Association for Rett Syndrome support (AIRETT, <http://www.airett.it/>) is gratefully acknowledged. The work has also been partially supported by the European Union Grant FINGER, QLG2-CT-1999-00920 (to AF).

**Gene expression profiling identifies RNA regulation defects in Amyotrophic Lateral Sclerosis muscle.** *K. Bouri*<sup>1</sup>, *D. Escolar*<sup>1</sup>, *R. Mandler*<sup>2</sup>, *C. Brandoli*<sup>1</sup>, *Y-W. Chen*<sup>1</sup>, *E. Pegoraro*<sup>3</sup>, *C. Angelini*<sup>3</sup>, *E.P. Hoffman*<sup>1</sup>. 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Department of Neurology, George Washington University, Washington, DC; 3) Department of Neurology and Psychiatric Sciences, University of Padova, Italy.

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease affecting upper and lower motor neuron and atrophy of affected muscles that leads to paralysis and finally death. We tested the hypothesis that muscle shows ALS specific changes not shared with other denervation or dystrophic states. In order to identify genes that are differentially expressed as a result of ALS, we performed expression profiling of 12,000 genes in ALS patients, juvenile ALS, Charcot-Marie-Tooth (CMT) and control muscles using oligonucleotide microarray (Affymetrix). Patients muscle samples were processed individually on a custom muscle chip (cnHuMuscleA, Affymetrix) and compared to normal control muscles. Most differentially-regulated genes are involved in established pathways of mitochondrial metabolism, nerve degeneration/regeneration processes and growth factor signaling. Interestingly, we identified a gene that is specifically down regulated in adult and juvenile ALS, this gene belongs to a family of RNA metabolism gene processes and ribosomal DNA transcription. We have also identified an ALS specific gene involved in L-Serine biosynthesis, which plays an important role in development and function of the central nervous system. We hypothesize that these genes are involved in stabilizing the neuromuscular junction, and a reduction of these genes in ALS would disrupt the integrity of the NMJ and the ability to reinnervate the muscle after mild denervation, which leads to further damage.

**Spastin, a AAA protein involved in autosomal dominant spastic paraplegia, interacts dynamically with microtubules.** *A. Errico, A. Ballabio, E.I. Rugarli.* TIGEM, Naples, Italy.

Hereditary spastic paraplegia (HSP) comprises a heterogeneous group of neurodegenerative diseases characterised by degeneration of the corticospinal tracts. The gene responsible for the most frequent form of autosomal dominant HSP (SPG4) encodes a 616 amino acid protein, spastin, belonging to the AAA proteins family. The AAA family is characterised by the presence of a highly conserved module with ATPase activity named AAA cassette. Among the members of the AAA family, spastin has higher homology with SKD1, a protein involved in endosomal trafficking, and katanin, responsible for microtubule severing at the centrosome. Interestingly, almost all the missense mutations found in spastin fall into the AAA functional domain. In order to study spastin biological function, we performed subcellular fractionation and immunofluorescence (IF) experiments on transfected cells. These experiments showed that spastin has a cytoplasmic localisation and that its overexpression leads to the formation of aggregates. Interestingly, when a low level of expression is obtained, the protein is localised in the region of the centrosome. To investigate if the ATPase activity could modulate the protein localisation, we produced constructs to express a truncated spastin, lacking the AAA cassette, or a missense mutation predicted to block ATP hydrolysis. IF experiments with these constructs revealed a characteristic filamentous pattern, partially co-localising with alfa-tubulin, and sensible to nocodazole treatment. Furthermore, the distribution of microtubules in transfected cells is altered. The overexpression of all the missense mutations found in patients gave similar results, leading us to postulate that these mutations affect ATP hydrolysis. In conclusion, we have demonstrated that spastin interacts dynamically with microtubules and that this interaction is regulated by ATP hydrolysis. Because of the homology with katanin, we are investigating a possible role of spastin in microtubules severing. These results have important implications for understanding pathogenesis of the axonal degeneration in HSP due to spastin mutations.



**Characterization of gigaxonin, the defective protein in Giant Axonal Neuropathy.** *P. Bomont, M. Koenig.* Institut de Genetique et de Biologie Moleculaire et cellulaire, Strasbourg, France.

Giant Axonal Neuropathy (GAN, OMIM 256850), a severe, autosomal recessive sensorimotor neuropathy affecting both the peripheral nerves and the central nervous system, corresponds to a generalized disorganization of the cytoskeletal intermediate filaments, with neurofilaments predominantly affected.

We localized the GAN locus on chr 16q24.1 (Eur J Hum Genet 2000, 8: 527-534), identified the defective gene by a bioinformatic approach, and cloned the full cDNA by screening a human brain cDNA library (Nature Genetics 2000, 26: 370-374). We have now identified 18 distinct mutations including nonsense mutations in consanguinous families, frameshift and missense mutations, distributed through the 11 exons of the gene. RT-PCR, done on multiple mouse tissues with the orthologue EST indicated ubiquitous expression.

We are now studying the function of the corresponding protein gigaxonin, and looking for its implication in the intermediate filaments organization. Gigaxonin is composed of an amino-terminal BTB/POZ domain followed by six Kelch repeats, which are predicted to adopt a b-propeller shape. No orthologue protein to gigaxonin was present in databases. Since the highest identities among the proteins carrying both a BTB and a Kelch domain ranged only from 22 to 27%, gigaxonin represents a novel and distinct member of this family. We produced both polyclonal and monoclonal antibodies raised against three different peptides and against the N-terminal part of the protein. Gigaxonin localized in the cytoplasm of both COS and HeLa cells in transfection experiments, as revealed by immunofluorescence. Gigaxonin did not colocalize with any of the intermediate filaments, microtubules or microfilaments networks. Experiments with the endogenous gigaxonin are in progress. We detected gigaxonin by western blot and by immunoprecipitation experiments. The search of the putative partners of gigaxonin should provide indications about its function.

**Nasu-Hakola disease: a molecular and neuropathological study identifying abnormal microglia.** K. DEGUCHI<sup>1</sup>, K. SUZUKI<sup>2</sup>, I. KITAJIMA<sup>3</sup>, K. OGOMORI<sup>4</sup>, J.R. LUPSKI<sup>5</sup>, D.L. ARMSTRONG<sup>1</sup>, K. INOUE<sup>5</sup>. 1) Pathology, Texas Children's Hospital, Houston, TX; 2) Psychiatry, Yokohama City Univ., Yokohama, Japan; 3) Lab Clinical Med, Toyama Medical and Pharmaceutical Univ., Toyama, Japan; 4) Psychiatry, Kyushu University, Fukuoka, Japan; 5) Human Molecular Genetics, Baylor College of Medicine, Houston, TX.

Nasu-Hakola disease is a rare autosomal recessive disorder characterized by presenile dementia and bone cysts. Most patients have been found in Finland and Japan. A founder mutation in *TYROBP*, also known as *DAP12*, was found in Finnish Nasu-Hakola disease patients and a different mutation was found in a Japanese patient. *DAP12* was originally identified as an activating co-receptor of natural killer cells. To further delineate genetic basis of Nasu-Hakola disease, we studied 8 independent Japanese families with Nasu-Hakola disease to search for a mutation in *TYROBP*. In 7/8 families, we found a homozygous 144delC mutation, which was the same as the one originally described. Haplotype analysis suggested a common founder for this mutation. Neuropathology of Nasu-Hakola disease has been recognized as a white matter degenerative disease with spheroids. The relationship between the gene mutation and the neuropathology has not been previously studied. We investigated the distribution *TYROBP* protein in the normal brain by immunohistochemistry. *TYROBP* was expressed predominantly in microglia in the white matter and in some neurons in the cortex. In the patient brains we characterized the microglial cells and observed them to be positive with RCA-1 and CD68 but almost negative with ionized calcium binding adaptor molecule-1(Iba-1). This contrasts with other leukodystrophies that we investigated (adrenoleukodystrophy and metachromatic leukodystrophy), in which microglia were positive with Iba-1. These findings suggest that a mutation in *TYROBP* results in brain pathology with altered reactivity in the microglial function.

**Clinical and molecular characterization of Alexander disease patients.** *J.R. Gorospe<sup>1</sup>, S. Naidu<sup>2</sup>, A. Johnson<sup>3</sup>, G. Raymond<sup>2</sup>, O. Ayuste<sup>4</sup>, V. Puri<sup>5</sup>, S. Jenkins<sup>6</sup>, R. Pedersen<sup>7</sup>, D. Lewis<sup>8</sup>, P. Knowles<sup>9</sup>, E. Hoffman<sup>1</sup>.* 1) Center for Genetic Medicine, Children's National Med Ctr, Washington, DC; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Department of Pathology, Albert Einstein College of Medicine, Bronx NY; 4) Downers Grove IL; 5) Norton Healthcare, Louisville KY; 6) Department of Neurology, Children's Hospital Oakland, Oakland CA; 7) Department of Pediatrics - Neurology Service, Tripler Army Medical Center, Honolulu HI; 8) Department of Pediatrics - Neurology Division, Duke University Medical Center, Durham NC; 9) T.C. Thompson Children's Hospital, Chattanooga TN.

Alexander disease is a slowly progressive childhood disorder of the CNS variably manifested by bulbar signs, seizures, ataxia, and macrocephaly. MRI commonly reveal predominantly frontal leukoencephalopathy. Until recently, diagnosis could only be afforded by brain biopsy, where the presence of Rosenthal fibers was considered diagnostic. Mutations in the GFAP gene were recently found in 10 biopsy-proven patients (Brenner et al., *Nature Genetics* 27: 117). Here, we studied 22 patients whose history, physical examination and MRI findings were suggestive of Alexander disease. Sequencing the entire coding region revealed 11 patients (50%) with mutations in GFAP. Age of onset in mutation-positive patients ranged from 2m to 9y. Infantile patients typically presented with seizures and macrocephaly, while older onset patients presented with variable symptoms, including normocephaly, intractable vomiting, and failure to thrive after a relatively unremarkable early childhood. With advancing age, patients developed speech abnormalities, dysphagia, cognitive deficits, and uncoordinated movements, with/without seizures. All the identified mutations involved sporadic single amino acid heterozygous changes: M73R (1), R79G (1), R79H (2), R88C (2), R239C (1), Y242D (1), E373X (1), R416W (2). Most were novel mutations, suggesting that complete screening of the GFAP gene is required for accurate diagnosis. In conclusion, GFAP mutation-positive Alexander disease patients show a variable clinical phenotype. Additionally, patients with a predominantly frontal leukoencephalopathy should be tested for mutations in the GFAP gene.

**Infantile Alexander Disease: spectrum of GFAP mutations and genotype-phenotype correlation.** *O. Boespflug-Tanguy*<sup>1</sup>, *D. Rodriguez*<sup>2,3</sup>, *F. Gauthier*<sup>1</sup>, *E. Bertini*<sup>4</sup>, *G. Uziel*<sup>5</sup>, *R. Surtees*<sup>6</sup>, *S. N'Guyen*<sup>7</sup>, *C. Goizet*<sup>8</sup>, *A. Gelot*<sup>2</sup>, *JM. Pedespan*<sup>8</sup>, *M. Troncoso*<sup>9</sup>, *M. Brenner*<sup>9</sup>, *A. Meesing*<sup>9</sup>, *G. Ponsot*<sup>2</sup>, *D. Pham Dinh*<sup>3</sup>, *A. Dautigny*<sup>3</sup>. 1) Fac de Medecine, Inserm U384, Clermont-Ferrand, France; 2) Hopital Saint Vincent de Paul, Paris; 3) URA CNRS 1488, Paris; 4) Ospitale Bambino Di Jesu, Roma, Italy; 5) Istituto Carlo Besta, Milano, Italy; 6) Institute of Child Health, University College, London, UK; 7) University Hospital Nantes, France; 8) University Hospital Bordeaux, France; 9) Hospital Clinico San Borja Arriaran, Santiago, Chile; U. Birmingham, Alabama and U. Wisconsin, Madison, USA.

Alexander disease (AD) is a leukoencephalopathy characterized by the presence of Rosenthal fibers (RF) in astrocytes. RF are cytoplasmic inclusions containing the intermediate filament protein GFAP. In infantile cases, the disease begins during the first 2 years of life with macrocephaly, psychomotor regression, seizures leading to death within the first decade. MRI is useful for diagnosis showing white matter signal changes with a rostrocaudal gradient, basal ganglia and thalamic abnormalities. Most of AD are sporadic. The genetic origin of this disease was still controversial when RF were found in the brain of mice overexpressing human GFAP. Heterozygous, de novo, GFAP missense mutations were found in 12 of 13 patients affected by a neuropathologically proven AD. We investigated a new series of 16 patients with heterogeneous clinical symptoms but neuroimaging abnormalities suggestive of AD. Missense, de novo, GFAP mutations, restricted to exons 1 and 4, were found in 15 cases including patients without macrocephaly and a slowly progressive white matter disease. Ten patients had previously described arginine mutations (5 R79H; 4 R239C and 1 R239H), but the other 5 had 3 novel mutations including arginine (2 R88C, 1 R88S) or non-arginine residues (L76F; N77Y), with a correlation according to the clinical severity. All mutations are located in the rod domain of the GFAP. These results confirm that dominant GFAP mutation is a reliable molecular marker for the diagnosis of infantile AD, including prenatal diagnosis to detect cases of germinal mosaicism.

**Ataxia with primary vitamin E deficiency (AVED): clinical, biochemical and molecular study in Italian patients.**  
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AVED is a rare autosomal recessive neurodegenerative disorder due to mutations in the  $\alpha$ -tocopherol transfer protein ( $\alpha$ -TTP) gene located on 8q13. AVED patients present an impaired ability to incorporate  $\alpha$ -tocopherol into VLDL secreted by the liver. Vitamin E is a potent lipid-soluble antioxidant, especially effective in scavenging peroxy radicals in cell membranes. In AVED patients, plasma levels of vitamin E are usually less than 10% of controls. The disease has a juvenile onset and is most frequent in North-African and Mediterranean populations. The neurological phenotype is very similar to that observed in patients affected with Friedreich's ataxia (FRDA), the most common hereditary ataxia. Unlike FRDA, however, cardiac involvement and impairment of glucose metabolism are very unusual in AVED patients. Progression of neurological deficits can be prevented by daily supplementation of high doses of vitamin E.

We studied 13 ataxic patients from 11 families of Italian origin, who exhibited very low plasma levels of vitamin E (range 0.11 - 0.33 mg%; nv,  $1.22 \pm 0.23$  mg%). Age at onset ranged from 4 to 27 y. None of them presented cardiomyopathy or diabetes. We identified pathogenic mutations in 21/22 alleles. Two mutations, 744delA and 513insTT, were found to be prevalent. These mutations accounted for 70% of the mutated alleles (16/22). The 744delA mutation had been previously described in the majority of patients of Mediterranean origin, while the 513insTT had been found in patients of North European origin. We also identified two novel mutations: a homozygous 210insTA identified in a patient from consanguineous parents and a Gly246Arg missense mutation found in a compound heterozygous patient carrying the 513insTT on the other allele. The characterization of AVED patients is particularly relevant because early treatment with vitamin E can delay the progression of the disease. Moreover, the treatment of presymptomatic subjects in characterized families could potentially prevent the development of neurological deficits.

**The effects of  $\alpha$ -synuclein expression on gene expression profiles in familial Parkinson's disease.** *B.A. Chase<sup>1</sup>, L. Avery<sup>2</sup>, Z.K. Wszolek<sup>3</sup>, K. Markopoulou<sup>2</sup>.* 1) Dept of Biology, Univ Nebraska, Omaha, NE; 2) Dept of Neurological Sciences, Univ of Nebraska Medical Center, Omaha, NE; 3) Dept of Neurology, Mayo Clinic, Jacksonville, FL.

Mutations in  $\alpha$ -synuclein (G209A, G88C) are associated with Parkinson's disease (PD). The function of normal  $\alpha$ -synuclein is presently unknown.  $\alpha$ -Synuclein is identified in aggregates - Lewy bodies (PD) and amyloid plaques (Alzheimer's disease), yet mutations have only been found in familial PD. In familial PD with the G209A mutation, there is considerable phenotypic variability including age of onset, disease severity and duration and neuropathology. We have found that in familial PD, the expression of the G209A allele is significantly reduced or absent. To gain insights into the role(s) of  $\alpha$ -synuclein and its expression status in neurodegeneration we used cDNA microarrays to profile gene expression as a function of G209A  $\alpha$ -synuclein allele expression status. We assayed the expression of approximately 3500 genes in cell lines established from members of a large kindred with PD. To assess the effects of differential  $\alpha$ -synuclein expression on the expression of other genes we compared symptomatic individuals not expressing the G209A allele to asymptomatic individuals who did not express the G209A allele, but were older than the mean age of disease onset for their generation, and to individuals who were asymptomatic, did express the G209A allele, and were younger than the mean age of onset for their generation. We find that the expression of a large number of genes is differentially affected as a function of the expression of the  $\alpha$ -synuclein mutant allele. Here, we focus on two sets of genes, one involved in energy and one in the proteasome pathway. For example, the expression of different subunits of the mitochondrial ATP synthase and NADH dehydrogenase, E3 ubiquitin ligase and ubiquitin conjugating enzyme E2 are overexpressed in the absence of the expression of the G209A allele. These findings support the hypothesis that the status of  $\alpha$ -synuclein gene expression plays a role in neurodegeneration and that the  $\alpha$ -synuclein gene functions in networks affecting multiple aspects of cellular function.

**A novel myelin protein zero mutation associated with Charcot-Marie-Tooth type II disease.** *E. Di Maria<sup>1</sup>, D. Cassandrini<sup>1</sup>, P. Balestra<sup>1</sup>, F. Manganelli<sup>2</sup>, L. Santoro<sup>2</sup>, F. Ajmar<sup>1</sup>, P. Mandich<sup>1</sup>, E. Bellone<sup>1</sup>.* 1) Dept. of Neurological Sciences and Vision, University of Genova, Genova, Italy; 2) University of Napoli Federico II.

Charcot-Marie-Tooth type II disease (CMT2) is a typical peroneal muscular atrophy syndrome and is characterised by normal or slightly reduced nerve conduction velocities with signs of axonal degeneration. CMT2 is genetically heterogeneous: linkage to 1p35-p36 (CMT2A), 3q (CMT2B) and 7p (CMT2D) loci has been reported for the autosomal dominant disease; however, the majority of CMT2 families do not link to any of the reported loci. Mutations of the myelin protein zero (MPZ) gene were found associated with demyelinating forms of hereditary neuropathies such as CMT1B, Dejerine-Sottas syndrome and congenital hypomyelination. So far, few CMT2 cases were found to be caused by point mutations in the MPZ (see CMT Mutation Database, <http://molgen-www.uia.ac.be/CMTMutations/>).

We report a family in which three members are affected with a late-onset peripheral neuropathy. The index patient is a 68 years old male who presents with pronounced distal muscle weakness of inferior limbs, bilateral *pes cavus* and absence of deep tendon reflexes. Electrophysiological findings were suggestive of an axonal form of peripheral neuropathy, thus allowing the diagnosis of CMT type 2. At the clinical and electrophysiological examination, two other family members (first cousins of the proband) resulted to be affected. MPZ gene direct sequencing revealed a heterozygous T/A transversion in the exon 3 of the gene, predicting an Asp103Glu aminoacid substitution in the extracellular domain of the protein. This variant was not found in unaffected relatives and in 100 normal chromosomes. This finding confirms the role of protein zero in axonal neuropathies and further suggests that the assessment of the MPZ gene should be included in the molecular diagnostic protocol of CMT2 patients.

(The laboratory is a member of the European CMT Consortium; partially granted by Ministero della Sanit to PM, MURST to FA).

**An hPer2 Phosphorylation Site Mutation in Familial Advanced Sleep-Phase Syndrome.** *Y. Fu*<sup>1</sup>, *C.R. Jones*<sup>2</sup>, *K. Toh*<sup>3</sup>, *D. Virshup*<sup>4</sup>, *L.J. Ptacek*<sup>5</sup>. 1) Neurobiology and Anatomy, Univ Utah, SLC, UT; 2) Department of Neurology, Univ Utah, SLC, UT; 3) Department of Human Genetics, Univ Utah, SLC, UT; 4) Department of Pediatrics, Univ Utah, SLC, UT; 5) Howard Hughes Medical Institute, Univ Utah, SLC, UT.

Advanced Sleep Phase Syndrome (ASPS) is manifest by early sleep preference and early morning awakening. It is very common in elderly people who tend to sleep and wake earlier than when they were younger. Familial advanced sleep phase syndrome is an autosomal dominant circadian rhythm variant that is similar although much more severe than the ASPS of aging; affected individuals are "morning larks" with a 4 hour advance of the sleep, temperature and melatonin rhythms. We now report localization of the FASPS gene near the telomere of chromosome 2q. A strong candidate gene (hPer2), a human homolog of the period gene in *Drosophila*, maps to the same locus. Affected individuals have a serine to glycine mutation within the casein kinase I (CKI) binding region of hPER2, that causes hypophosphorylation by CKI in vitro. Thus, a variant in human sleep behavior can be attributed to a missense mutation in a clock component, hPER2, that alters the circadian period. We have now expanded our collection of families and are looking for other hPer2 gene mutations. In addition, other candidate circadian rhythm genes are being screened for mutations in probands who don not have hPer2 mutations.



**Gene Expression Profiling in Infantile SMA Muscle.** *C. Brandoli<sup>1</sup>, K. Bouri<sup>1</sup>, D.M. Escolar<sup>1</sup>, Y-W. Chen<sup>1</sup>, S. Servidei<sup>2</sup>, E.P. Hoffman<sup>1</sup>.* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Institute of Neurology, Catholic University of Rome, Italy.

Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disease occurring 1 in 10,000 newborns. While the primary pathological feature of SMA has long been considered to be the loss of the cell bodies of a-motor neurons in the anterior horn of the spinal cord, increasing evidence points to additional primary defects of the end target of the motor neurons, namely skeletal muscle. SMA is caused by reduction of the expression of the survival of motor neuron (SMN) gene, which is thought to regulate a series of downstream mRNAs, which have not yet been defined. We used gene expression profiling of biopsies from infantile SMA patients to define the pathophysiological mechanisms involved in the onset and progression of the disease. Expression profiling was done on five muscle biopsies from SMA type I patients and compared to age/sex matched normal controls. 70 genes of 15,000 studied showed more than 2-fold-up or down regulation in SMA patient muscle. The most interesting group of down-regulated genes involved transcription factors with 24-fold under expression of a specific Zinc finger protein; similar proteins have recently been shown to interact with SMN and co-localizes with SMN in subnuclear structures. We found a number of genes, typically overexpressed during denervation, which were either downregulated (c-fos, jun-B, NF-IL6) or upregulated (IGF-II) in SMA muscle. We developed a model where nerve/muscle interaction is disrupted in SMA, where some compensatory re-innervation signaling pathways are lost due to SMN depletion, while other pathways are retained but insufficient to sustain or re-establish innervation. Our finding of dramatic down-regulation of a Zinc finger protein suggests that this protein fails to associate with gems, inhibits splicing or activation of other transcription factors which are up-regulated during normal muscle denervation. The loss of muscle molecular response triggers a secondary mechanism of re-innervating signals to the nerve: degenerating motor neurons are unable to respond to the signals and inappropriate cell death occurs.

***Atlastin* gene analysis in early onset hereditary spastic paraplegia.** D.M. Alvarado<sup>1</sup>, L. Ming<sup>1</sup>, P. Hedera<sup>1</sup>, S. Rainier<sup>1</sup>, X. Zhao<sup>1</sup>, W. Raskind<sup>2</sup>, T. Bird<sup>3</sup>, J.K. Fink<sup>1,4</sup>. 1) Department of Neurology, University of Michigan, Ann Arbor, MI; 2) Department of Medicine, University of Washington, Seattle, WA; 3) Department of Neurology, University of Washington, Seattle, WA; 4) Geriatric Research Education Clinical Center, Ann Arbor Veterans Affairs Medical Center.

Hereditary spastic paraplegia (HSP) is a group of disorders characterized by progressive lower extremity spastic weakness. 7 loci for "uncomplicated" autosomal dominant HSP (ADHSP) have been identified. These forms of "uncomplicated" HSP are clinically similar although differ in the average age of symptom onset. Symptoms begin before age 11 yrs.(on average) in ADHSP linked to chr. 12 (SPG10), 14 (SPG3), and 19 (SPG12); and after age 20 yrs. (on average) in ADHSP linked to chr. 2p (SPG4), 2q (SPG13) 8 (SPG8), and 15(SPG6). Recently (*Zhao et al, ASHG abstract 102*), we discovered disease-specific missense mutations in a novel GTPase (designated *atlastin*) in each affected subject from 3 families with chr.14q-linked ADHSP in which symptoms began before age 10 years.

In the present study, we investigated the frequency of *atlastin* gene mutations in childhood onset ADHSP. We sequenced each *atlastin* exon in affected subjects from 11 ADHSP kindreds in which the disorder began in childhood. Small family size (9 kindreds)precluded linkage analysis; 1 early onset ADHSP kindred had lod score to the SPG3 locus »1.0. We also analyzed samples from subjects from 2 small ADHSP kindreds with later symptom onset (6-52 years) but for whom lodscore = 1.0 suggested possible linkage to the chr. 14q SPG3 locus.

We found disease-specific *atlastin* mutations in 2 of 10 early onset ADHSP kindreds that were not pre-selected for linkage to SPG3. Both families had the same mutation (codon 239 Arg@Cys) which was previously identified in affected subjects from an ADHSP kindred linked to the SPG3 locus. These observations suggest that 20% of early onset ADHSP kindreds have *atlastin* coding sequence mutations.

**Missense mutation in TSC2 associated with an unusually mild form of tuberous sclerosis.** *D. D'Agostino<sup>1</sup>, F. Dubeau<sup>1</sup>, A. Al-Asmi<sup>1</sup>, S. Bourgoin<sup>2</sup>, M. Labuda<sup>2</sup>, S. Mercho<sup>1</sup>, F. Andermann<sup>1</sup>, M. Pandolfo<sup>2</sup>, D.J. Kwiatkowski<sup>3</sup>, E. Andermann<sup>1</sup>.* 1) Montreal Neurological Institute and Hospital, McGill University, Montreal, PQ, Canada; 2) Centre Hospitalier de l'Université de Montréal, Montréal, PQ, Canada; 3) Brigham and Women's Hospital, Boston, Harvard University MA.

Tuberous sclerosis is an autosomal dominant disorder characterized by hamartomata in various organs, including skin, brain and kidneys, caused by TSC1 (9q34) or TSC2 (16p13.3) mutations. Familial cases are equally distributed between TSC1 and TSC2. The majority of sporadic patients have TSC2 mutations. The disease phenotype is milder in sporadic TSC1 patients (Dabora et al., 2001).

We report a large extended kinship with exceptionally mild TSC2.

Field trips were carried out to examine the family members and to collect blood samples. We reviewed medical records and brain imaging data (MRI). 55 subjects were genotyped for markers linked to the loci for familial partial epilepsy with variable foci (22q11), TSC1 (9q34) and TSC2 (16p13). We defined the affected status by coexistence of epilepsy and skin lesions. Two point lod scores were calculated using the MLINK program.

Of 65 individuals examined, 18 had epilepsy. In most patients, seizures were well controlled by medication and became milder or resolved at older ages. All assessed individuals with epilepsy had hypopigmented macules on the limbs and trunk. An additional 12 family members had only hypopigmented macules. No facial angiofibromas, periungual fibromas or retinal hamartomas were found. MRI, available in 7 patients, showed left frontal sub-ependymal giant cell astrocytoma and sub-ependymal nodules in one patient each. 1/5 screened individuals had renal angiomyolipoma. No rhabdomyoma was revealed by echocardiography. Penetrance of epilepsy was about 60%. The penetrance of hypopigmented macules reached almost 100%. Linkage to the TSC2 locus was demonstrated. Mutation analysis revealed a missense mutation in exon 23 of the TSC2 gene (2714G®A, 905R®Q).

This study should contribute to clarifying genotype-phenotype correlations in familial TSC2.

**Phenotypic consequences of mosaic MeCP2 high expression in cerebral cortical neurons: A quantitative comparison of normal and Rett brains.** *J.M. LaSalle<sup>1</sup>, J. Goldstine<sup>1</sup>, D. Balmer<sup>1</sup>, C.M. Greco<sup>2</sup>*. 1) Medical Microbiol & Immunology, Univ California Davis Sch Med, Davis, CA; 2) Medical Pathology, UCDCM, Sacramento, CA.

Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder caused by mutations in *MECP2*, encoding the methyl-CpG-binding protein 2 (MeCP2). The role of MeCP2 in postnatal neuronal development is currently unknown. We have recently developed an approach for quantitating the level and distribution of wild-type and mutant MeCP2 *in situ* by immunofluorescence and laser scanning cytometry. Surprisingly, cellular heterogeneity in MeCP2 expression level was observed in normal brain with a subpopulation of cells exhibiting high expression (MeCP2<sup>hi</sup>) and the remainder exhibiting low expression (MeCP2<sup>lo</sup>). MeCP2 expression was significantly higher in CNS compared to non-CNS tissues of human and mouse by automated quantitation of MeCP2 on multiple tissue arrays. Quantitative localization of MeCP2 expression phenotypes in normal human brain showed a mosaic but distinct distribution pattern, with MeCP2<sup>hi</sup> neurons highest in layer IV of the cerebrum. Using combined fluorescent staining approaches for neurons (fluorescent Nissl dye), chromatin (propidium iodide), nucleoli (anti-nucleolin), and mRNA (oligo dT hybridization), quantitation of phenotypic differences between MeCP2<sup>lo</sup> and MeCP2<sup>hi</sup> neurons was determined in normal human cerebral sections. MeCP2<sup>hi</sup> neurons had significantly larger nuclei with more euchromatin, larger nucleoli, and more transcription when compared to MeCP2<sup>lo</sup> neurons. In contrast, no difference was observed in the level of histone acetylation or Nissl stain between the two populations. Using a C-terminal specific anti-MeCP2 to identify neurons expressing the truncated allele of *MECP2* in RTT cerebrum, mutant-expressing neurons were found to have the phenotypic characteristics of MeCP2<sup>lo</sup> neurons. Use of an N-terminal specific anti-MeCP2 showed normal ratios of MeCP2<sup>hi</sup> cells in RTT brains, demonstrating normal expression of mutant MeCP2. These results demonstrate that MeCP2 mutations in RTT are primarily manifested in phenotypically MeCP2<sup>hi</sup> cells and suggest that MeCP2 loss-of-function inhibits chromatin organization and activation of cerebral neurons.

**Abnormal processing of proteolipid protein pre-mRNA is associated with an unusual presentation of Pelizaeus-Merzbacher disease.** *G.M. Hobson<sup>1</sup>, Z. Huang<sup>2</sup>, A. McCullough<sup>3</sup>, H.G. Marks<sup>4</sup>, D. Stabley<sup>1</sup>, K. Sperle<sup>1</sup>, F. Cambi<sup>2</sup>.* 1) Dept Research, Alfred I duPont Hosp Children, Wilmington, DE; 2) Dept Neurology, Thomas Jefferson University School of Medicine, Philadelphia, PA; 3) Dept Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX; 4) Dept Pediatrics and Neurology, University of Pennsylvania Medical School, Philadelphia, PA.

Mutations in the proteolipid protein gene (*PLP1*) are associated with X-linked disorders that span in clinical severity from the severe developmental phenotype of Pelizaeus-Merzbacher disease (PMD) to mild forms of pure hereditary spastic parapareses (HSP). Genetic determinants of the phenotypic variability remain unclear. Two proteins, PLP and DM20, are generated from the same primary transcript by alternative splicing of intron 3, which is regulated as part of oligodendrocyte differentiation. We identified a deletion of a novel G-rich element within intron 3. The proband was affected initially by a very mild phenotype, but he lost motor and cognitive milestones in the end of his first decade of life, a rapidly progressive course not commonly observed in PMD. Early MRI studies demonstrated normal appearance of white matter, suggesting relatively normal myelin deposition; however, subsequent MRI and MRS studies showed a significant degree of axonal loss and some myelin breakdown. The imaging findings correlate with the clinical course of his disease. Our molecular studies show that PLP-specific splicing is impaired in CG4 cells and primary rat oligodendrocytes transfected with a chimeric construct carrying the 19-nucleotide deletion. DM20 transcript is expressed normally, suggesting that the deletion results in drastic reduction of PLP transcript and protein. In cross-linking experiments in vitro, we detected binding of a protein factor to the 19-nucleotide sequence. We conclude: 1) regulatory elements in the 19-nucleotide sequence participate in selection of splicing and cell-specific expression of the PLP transcript, 2) alterations in RNA processing that result from the deletion lead to the selective loss of PLP, and 3) the drastic reduction in PLP may affect myelin stability and axon-glia interactions resulting in axonal loss.

**Phenotypical variability in loci of holoprosencephaly.** *S. Odent*<sup>1</sup>, *M. Blayau*<sup>2</sup>, *L. Lazaro*<sup>2,3</sup>, *A. De La Pintiere*<sup>2</sup>, *C. Aguilera*<sup>2</sup>, *L. Pasquier*<sup>1</sup>, *C. Dubourg*<sup>2</sup>, *V. David*<sup>2,3</sup>. 1) Genetique medicale, Hopital Pontchaillou, Rennes, France; 2) Genetique molculaire, Hopital Pontchaillou, Rennes, France; 3) UPR41 CNRS, Faculte de medecine, Rennes, France.

Holoprosencephaly (HPE ; 1/16.000 live births ; 1/250 conceptuses) is a common development defect affecting both the forebrain and the face. Clinical expressivity is variable, ranging from a single cerebral ventricle and cyclopia to clinically unaffected obligated carriers in familial HPE. The disease is genetically heterogeneous but additional environmental agents also contribute to the aetiology of HPE. This study includes 121 unrelated nonchromosomal HPE cases (75 typical HPE, 28 atypical cases, 18 polymalformative cases). We provide clinical data regarding the subgroup of typical HPE and report 18 novel heterozygous mutations (15% for all the cases, 22% for typical HPE), 10 in Sonic hedgehog gene (SHH), 4 in ZIC2, 3 in SIX3, and 1 in TGIF. Nine mutations were found in familial cases whereas 9 mutations were identified in apparently sporadic cases. Original phenotypes associated with a mutation have been observed : isolated cleft lip and palate, abnormalities in the pituitary gland and corpus callosum, colobomatous microphthalmia and brachymetacarpia, choanal stenosis, without HPE. This study confirms the extremely variable phenotypes in HPE families and the genetic heterogeneity of the disease.

**Compensating for CNS dysmyelination: females with a *PLP* duplication and sustained clinical improvement.** *K. Inoue*<sup>1</sup>, *H. Tanaka*<sup>2</sup>, *F. Scaglia*<sup>1</sup>, *A. Araki*<sup>2</sup>, *L.G. Shaffer*<sup>1</sup>, *J.R. Lupski*<sup>1</sup>. 1) Molec & Human Genetics, Baylor College Medicine, Houston, TX; 2) Pediatrics, Asahikawa Habilitation Center for Disabled Children, Asahikawa, Japan.

A submicroscopic duplication in Xq22 that contains the entire proteolipid protein gene (*PLP*) is the major cause of Pelizaeus-Merzbacher disease (PMD), a central nervous system (CNS) dysmyelinating disorder. Previous studies revealed that carrier females for the duplication are usually asymptomatic. In this study we describe two unrelated female patients who present with mild PMD or spastic paraplegia. In one patient, the clinical features, along with findings on cranial magnetic resonance imaging and brainstem auditory evoked potentials, have improved dramatically over a ten year period. The other patient, who presented with spastic diplegia and was initially diagnosed with cerebral palsy, has also had clinical improvement. Interphase fluorescent in situ hybridization (FISH) identified a *PLP* duplication in both patients. FISH analyses of the family members indicated that the duplication in both patients occurred as de novo events. Neither skewing of X inactivation in the peripheral lymphocytes nor *PLP* coding alterations were identified in either patient. These findings indicate that, in rare cases, females with a *PLP* duplication can manifest an early onset neurological phenotype. We hypothesize that the remarkable clinical improvement is due to myelin compensation by oligodendrocytes expressing one copy of *PLP* secondary to selection for a favorable X inactivation pattern. These findings indicate plasticity of oligodendrocytes in the formation of the CNS myelin and suggest a potential role for stem cell transplantation therapies.

**Expression map of the DYT3 critical region in Xq13.1.** *U. Muller<sup>1</sup>, S. Niemann<sup>1</sup>, J. Ramser<sup>2</sup>, H. Lehrach<sup>3</sup>, R. Sudbrak<sup>3</sup>, D. Nolte<sup>1</sup>.* 1) Inst Human Genetics, Justus-Liebig Univ, Giessen, Germany; 2) Medical Genetics, LMU Munic, Germany; 3) MPI Molecular Genetics, Berlin, Germany.

The X-linked dystonia parkinsonism syndrome (XDP) is a severe movement disorder characterized by dystonia and by parkinsonism in about 50% of cases. Due to the origin of all XDP cases from an ancestral founder, the disease locus, DYT3, could be assigned by analysis of allelic association to a small interval of approximately 300 kb in Xq13.1. This interval is flanked by markers DXS559 distally and by DXS6673E (3) proximally. In search of the disease gene we have constructed an expression map of the region. There are at least seven expressed genes. They are - from proximal to distal - DXS6673E, a gene of unknown function, NonO coding for p54nrb (a nuclear RNA binding protein), a gene coding for muscle-specific melusin (that interacts with integrin), CCG1 coding for TATA binding protein-associated factor TAFII250, ING IL, a putative tumor suppressor gene that is a member of the p33INGI gene family, OGT the gene for O-linked N-acetylglucosamine transferase, NAAR1 coding for a nuclear acidic amino acid repeat containing nuclear protein, and CRK-L2 that codes for a G-protein coupled receptor. One of these genes, NAAR1 (nuclear acidic amino acid repeat 1) has not been recognized before. NAAR1 codes for a polypeptide with an acidic repeat tract of 21 units of 8-10 amino acids that has not been found in any other known protein. Several NLSs suggest nuclear location of the protein. Expressed portions of all genes were analyzed for mutations in XDP patients. The absence of a mutation in expressed sequences of these genes suggests that XDP is caused by either a mutation in a regulatory region or by a structural rearrangement.



**Mutational analysis in early-onset parkinsonism (EOP) including gene dosage studies.** *K. Hedrich<sup>1,2</sup>, J. Harris<sup>3</sup>, M. Kann<sup>1,2</sup>, T. Lynch<sup>3</sup>, H.M. Santana<sup>3</sup>, E. Schwinger<sup>1</sup>, P.P. Pramstaller<sup>4</sup>, S.B. Bressman<sup>5</sup>, S. Fahn<sup>3</sup>, K. Marder<sup>3</sup>, C. Klein<sup>1,2</sup>.* 1) Department of Human Genetics, Medical University of Luebeck, Luebeck, Germany; 2) Department of Neurology, Medical University of Luebeck, Luebeck, Germany; 3) Department of Neurology, Columbia University, New York, NY, USA; 4) Department of Neurology, Regional General Hospital, Bolzano, Italy; 5) Department of Neurology, Albert Einstein College of Medicine, Bronx, NY, USA.

Autosomal recessive EOP is a progressive neurodegenerative disorder which is clinically similar to idiopathic Parkinson's disease but may be associated with additional neurological signs and usually starts before the age of 50 years. EOP has been associated with different mutations in the parkin gene, including exon deletions and duplications. Heterozygous exon deletions and duplications (gene dosage alterations) are difficult to detect by conventional screening methods. To test for gene dosage alterations, we developed a new method of quantitative duplex PCR using the fluorescence resonance energy transfer (FRET) technique on the LightCycler. We co-amplified beta globin as internal standard with each individual exon of parkin. In this study, 37 isolated patients with EOP (mean age of onset  $39.3 \pm 9.1$  yrs.) and 13 EOP patients (mean age of onset  $34.5 \pm 14.1$  yrs.) ascertained by positive family history were collected. Among these 50 patients, we detected 8 alterations of gene dosage, including heterozygous deletions of exons 1, 2, 3, and 3-4 and a heterozygous duplication of exon 5. In addition, 12 mutations (eight missense mutations, a silent single basepair substitution, and three small deletions) and known polymorphisms in exons 2, 4, 8, 10, and 11 were detected by SSCP and sequence analysis. In total, we identified 7 compound heterozygous mutation carriers (14%), 6 with heterozygous mutations (12%), and 37 with no detected mutation (74%) in the parkin gene. Taken together, we performed the first study systematically screening all 12 exons of parkin by real-time, kinetic quantification, an accurate method to detect heterozygous gene dosage alterations in parkin. Genetic studies of EOP must therefore include gene dosage alteration detection.

**Giant Axonal Neuropathy (GAN) caused by mutations in the Gigaxonin gene.** *G. Kuhlenbaumer<sup>1</sup>, P. Young<sup>1</sup>, C. Oberwittler<sup>2</sup>, B. Ringelstein<sup>1</sup>, F. Stogbauer<sup>1</sup>.* 1) Neurology, University of Muenster, Muenster, Westfalia, Germany; 2) Neurology, St. Vincenz Krankenhaus, Limburg, Germany.

Giant Axonal Neuropathy (GAN, MIM256850) is a severe autosomal recessively inherited neuropathy. The age of onset is usually below seven years. In most cases motor milestones are delayed. Later clumsiness of gait and progressive weakness becomes apparent. Tendon reflexes are usually absent and pyramidal signs are positive. Subsequently dysarthria, nystagmus, facial weakness and mental retardation develop. Patients have characteristic "frizzly" hair. Electrophysiological findings are typical for axonal neuropathies. Pathologic studies demonstrated that GAN is a disorder of intermediate filaments. Recently disease causing mutations were found in the gigaxonin gene on chromosome 16q24.1. It has 11 exons and encodes a protein of 597 amino acids. Gigaxonin belongs to the BTB/kelch repeat gene family and is ubiquitously expressed. Most BTB/kelch repeat proteins play a role in the formation of the cytoskeleton. We describe clinically and genetically a child with GAN and confirm for the first time that the disease is caused by mutation in the gigaxonin gene. The propositus is a 12 year old boy with grey, characteristically curled hair. He started to walk at 2 years. Somewhat later he developed progressive symmetrical weakness predominantly affecting the lower limbs, distal sensory impairment, ataxia and nystagmus. His school performance started to deteriorate in the last couple of years and since 1999 he is wheelchair bound. The electrophysiological examination showed signs of a severe axonal neuropathy and the EEG was slowed. DNA sequencing of the protein coding region of the gigaxonin gene revealed a stop mutation in exon 3 (C601T; Arg201Stop) on one allele and a missense mutation in exon 8 (T1268C; Ile423Thr) on the other allele. The mutation in exon 3 leads to truncation of the protein. The missense mutation is located in the kelch repeat region. Both mutations were not detected in 180 control chromosomes. The stop mutation in exon 3 was inherited from the father and the missense mutation in exon 8 from the mother.

**Mutations in the paraplegin gene (*SPG7*) in patients with sporadic or autosomal recessive spastic paraplegia are associated with a clinically distinct phenotype.** C. Mariotti<sup>1</sup>, S. Baratta<sup>1</sup>, G. Casari<sup>2</sup>, C. Milanese<sup>1</sup>, S. DiDonato<sup>1</sup>, C. Gellera<sup>1</sup>, L. Morandi<sup>1</sup>, L. Chiapparini<sup>1</sup>, F. Taroni<sup>1</sup>. 1) Divisione di Biochimica e Genetica, Istituto Neurologico C. Besta, Milan, Italy; 2) TIGEM, Milan-Neaples, Italy.

Hereditary spastic paraplegias (HSP) are a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by progressive weakness and spasticity of the lower limbs. They are clinically classified into “pure” and “complicated” forms (Harding, 1981). To date, 8 loci for autosomal dominant HSP, 3 loci for X-linked HSP, and 4 loci (*SPG5*, *SPG7*, *SPG11*, and *SPG14*) for autosomal recessive HSP (AR-HSP) have been identified. Pathogenic mutations in the *SPG7* gene, which is composed of 17 coding exons and encodes a novel mitochondrial metalloprotease named paraplegin, have been thus far demonstrated in 3 families with AR-HSP (Casari, 1998). We have analyzed the coding sequence (17 exons) of the paraplegin gene in 18 unrelated index cases, including 12 isolated cases and 6 cases with an autosomal recessive pattern of inheritance. An additional group of 10 patients have been tested for mutations in exons 2, 3 and 11. Mutation screening was carried out by direct automated sequencing of paraplegin exons and flanking intron sequences. We have identified 1 frameshift, 1 nonsense and 2 missense mutations in 5 out of 28 patients (18%). In our *SPG7*-mutated patients, age at onset ranged from 25 to 55 years. Clinical presentation was characterized by weakness and spasticity of the lower limbs associated with cerebellar signs such as gait ataxia and dysarthria. Cerebellar involvement was also clearly demonstrated by MRI. Despite the mitochondrial localization of the defective protein, proton-resonance spectroscopy of the brain did not show any metabolic abnormality. In conclusion, (1) the data would indicate that the frequency of mutations in the paraplegin gene might be higher than previously observed; (2) paraplegin mutations appear to be predominantly associated with a “complicated” phenotype.

**Frequency of Spastin mutations in German pedigrees with hereditary spastic paraplegia.** *S. Klimpe<sup>1</sup>, A. Visbeck<sup>1</sup>, D. Boensch<sup>2</sup>, H.C. Hopf<sup>1</sup>, T. Deufel<sup>2</sup>.* 1) Dept. of Neurology, Mainz, Germany; 2) Dept. of Clinical Chemistry, Jena, Germany.

**Introduction:** The Hereditary Spastic Paraplegias (HSP) comprise a genetically heterogeneous group of neurodegenerative disorders characterised by progressive spastic gait disorder and lower limb hyperreflexia; fifteen genetic loci have been described so far, with the SPG4 locus on chromosome 2p21-22 covering 40-50% in autosomal dominant families. The SPG4 gene has been identified and encodes for spastin, an AAA protein of unknown function. Mutation analysis has revealed all types of mutations in SPG4 patients. **Aim:** We screened German HSP families for spastin mutations to obtain prevalence data in this patient population. **Patients and Methods:** 16 families with 142 family members were assessed, 13 presented an autosomal dominant and three an autosomal recessive mode of inheritance. In addition, five patients with autosomal dominant family history but no familial blood samples were included. Inclusion criteria were according to Harding. Whenever there was a likelihood of autosomal dominant inheritance, segregation analysis for SPG4 was performed using polymorphic markers D2S352, D2S2283, D2S2347, D2S375 and D2S2351. In cases with positive LOD scores ( $>.0$ ), this was followed by direct sequencing of the spastin gene; additional cDNA sequencing was possible in four families. **Results:** In families with pure autosomal dominant HSP co-segregation with SPG4 was established in 11 pedigrees with LOD scores ranging from 0,3 to 2,7; in two families SPG4 could be excluded. In two pedigrees with LOD scores  $>2,0$  we detected spastin mutations that were present in all affected and in clinically possibly affected family members; cDNA sequencing revealed one splice site mutation and one single base deletion (exon 6, 1004delC, PTC +24AA), and genomic sequencing lead to the identification of the respective mutations. The splice site mutation leads to the deletion of exon 8, the single base deletion should result in a truncated protein. **Conclusions:** We identified spastin mutations in two out of 16 German pedigrees with pure autosomal dominant HSP families with possible linkage to the SPG4 locus. Ongoing sequencing of single patients samples may reveal further mutations.

**Analysis of SMAD2 as a candidate gene for holoprosencephaly.** *A.K. Kantipong<sup>1</sup>, J.D. Karkera<sup>2</sup>, E. Roessler<sup>2</sup>, M. Muenke<sup>2</sup>.* 1) HHMI-NIH Scholar, MGB, NHGRI/NIH, Bethesda, MD; 2) MGB, NHGRI/NIH, Bethesda, MD.

Genetic defects in all components of the Nodal signaling pathway including its signaling factors, receptors, cofactors and transcription factors have all been associated with the establishment of left-right axis symmetry and/ or cyclopia. In particular, SMAD2, an intracellular mediator of Nodal signaling, has been implicated in the development of cyclopia and defective left-right axis patterning in murine knockout studies. Holoprosencephaly (HPE) is characterized by a defect in the midline of the embryonic forebrain due to failure of growth and / or segmentation of the anterior end of the neural tube and is phenotypically similar to cyclopia. To determine whether sequence variants in SMAD2 can be associated with HPE, mutation scanning using PCR-dHPLC was conducted in a panel of over 400 patients (83 familial, and 350 sporadic). We have identified a silent variant (GA; Serine) in exon 8 and a deletion of two thymidines in the 5' intronic region of exon 10 but no obvious disease causing mutations. These variants were not found in our panel of normal controls (~200 chromosomes) suggesting that these are novel sequence variants. These data does not suggest a shift in fundamental human biology compared with animal systems but might be explained by the fact that 95% of HPE cases die in utero. Thus, mutations in SMAD2 which may be present in HPE fetuses might not be represented in our live-born sample set.

**Mutation analysis of the CX26 gene in sporadic cases with moderate to profound hearing impairment.** *S. Kupka<sup>1</sup>, T. Toth<sup>2</sup>, S. Aberle<sup>3</sup>, N. Blin<sup>3</sup>, I. Sziklai<sup>2</sup>, HP. Zenner<sup>1</sup>, M. Pfister<sup>1</sup>.* 1) Otolaryngology, University of Tuebingen, Tuebingen, BW, Germany; 2) Otolaryngology, University of Debrecen, Hungary; 3) Molekulare Genetik, University of Tuebingen, Tuebingen, BW, Germany.

Non-syndromic neurosensory recessive deafness (NSRD) is one of the most common human sensory disorders. Mutations in the connexin 26 gene have been established as a major cause of inherited and sporadic non-syndromic deafness in different populations. The CX26 gene encodes the gap junction protein connexin 26 (beta-2, GJB2) whose expression was shown in several tissues and in the cochlea. The 30delG mutation is the most frequent mutation in the CX26 gene. It represents a deletion of a guanosine (G) in a sequence of six G extending from position 30 to 35 of the CX26 cDNA. The deletion creates a frameshift resulting in a premature stop codon and a non-functional intracellular domain in the protein. The 30delG mutation can be detected at the molecular level using PCR followed by BsiYI-digestion. We now screened 100 control individuals and 250 patients with non-syndromic sporadic deafness for this mutation to determine their distribution in the German and Hungarian populations. The frequency of the 30delG mutation in the German pool of sporadic cases was 0.11 whereas in controls it was 0.04. While studying 24 small-sized Hungarian families, this frequency was 0.38. DNA from individuals showing a heterozygous status for 30delG was sequenced. This study revealed several new patient-related mutations and new gene variants resulting in e.g. amino acid substitutions (A->G; basic to acidic; G->A; nonpolar to polar; A->C; acidic to nonpolar). Moreover, one deletion and one insertion was noted. In summary, more than 20 new allelic changes were detected and for most of them, patterns of inheritance were documented.

**Investigation of the role of *MECP2* in the genetics and molecular pathology of Rett Syndrome.** A.M. Hever<sup>1</sup>, A. McDonnell<sup>1</sup>, M.T. Gardiner<sup>1</sup>, M. Ennis<sup>1</sup>, C. Johnstone<sup>1</sup>, A.M. Kerr<sup>2</sup>, G.J.R. Brock<sup>1</sup>, M.E.S. Bailey<sup>1</sup>. 1) Div. of Molecular Genetics, I.B.L.S., Univ. of Glasgow, Glasgow, U.K; 2) Dept. of Psychol. Medicine, Univ. of Glasgow, Glasgow, U.K.

Rett Syndrome is a severe neurodevelopmental disorder, inherited in an X-linked, dominant fashion and now known to be caused by loss-of-function mutations in the methyl CpG binding protein 2 gene, *MECP2*, located in chr.Xq28. The molecular pathology of the disorder is still very unclear, partly because the precise role of MECP2, which binds methylated CpG dinucleotides, has not been elucidated - it may play roles in regulation of tissue-specific genes, imprinting and suppression of mobile elements, the so-called genome defence hypothesis. Several other phenotypes are now known to be caused by mutations in the same gene, while not all patients with classic Rett syndrome harbour mutations in *MECP2*. We have investigated the molecular genetics of Rett Syndrome in 30 patients with the classic form and in 70 patients with atypical Rett syndrome. We have used intronic primers to screen patient DNA by PCR for mobility variants within exons 1, 2, 3, and coding portions of exon 4 using dHPLC technology, and then for mutations by direct sequencing and cloning. We have found mutations in more than 80% of patients with classic Rett syndrome, including several novel mutations. We are also examining the long 3'-UTR region, using multiple sets of overlapping primers, in those patients in whom no mutations were detected elsewhere. We have also investigated whether MECP2 has preferred binding sites in the genome by constructing an unbiased library of methyl binding domain (MBD)-binding genomic fragments using an MECP2 MBD binding column. Clones from this library have been identified by hybridisation to various classes of probe specific for different genomic DNA classes, followed by confirmatory sequencing of representative clones. Preliminary results have indicated that Alu repeats may be overrepresented in the library. We have therefore begun to investigate whether varieties of mobile element are overexpressed in Rett syndrome patients to try to determine the first step in the pathophysiology of Rett syndrome.

**A novel mutation of the sodium channel SCN1A in the epilepsy syndrome GEFS+.** B.T. MacDonald<sup>1</sup>, A. Escayg<sup>1</sup>, J. Spanpanato<sup>2</sup>, E. Montalenti<sup>3</sup>, P. Benna<sup>3</sup>, A.L. Goldin<sup>2</sup>, M.H. Meisler<sup>1</sup>. 1) Department of Human Genetics, University of Michigan, Ann Arbor, Michigan, USA; 2) Department of Microbiology and Molecular Genetics, University of California, Irvine, California, USA; 3) Department of Neuroscience, University of Torino, Turin, Italy.

Mutations of the voltage-gated sodium channel SCN1A on chromosome 2q24 have been identified in two epilepsy syndromes, Generalized Epilepsy with Febrile Seizures Plus (GEFS+) (MIM#604233) (Escayg et al., *Nat. Genet.* 24:343-345,2000) and Severe Myoclonic Epilepsy of Infancy (Claes et al., *Am. J. Hum. Genet.* 68:1327-1332,2001). GEFS+ is an autosomal dominant disorder characterized by fever-induced (febrile) seizures that persist beyond 6 years of age and progress to a variety of afebrile seizures. We previously described the effects of two GEFS+ mutations on SCN1A activity (Spanpanato et al, *J. Neurosci*, in press). Here we report the identification of a novel SCN1A mutation in an Italian family with GEFS+. In affected individuals in this family, febrile seizures persist to 12 years of age and progress to general tonic-clonic seizures. One child exhibited febrile seizures, myoclonic-astatic epilepsy, learning difficulties and behavioral problems. The exons of SCN1A were amplified from genomic DNA and screened by conformation-sensitive gel electrophoresis. A novel missense mutation was identified in an evolutionarily-invariant residue of the C-terminal domain. Protein structure predictions suggest that this mutation would change the boundary of an alpha-helical domain. Preliminary functional studies in *Xenopus* oocytes detected a reduced rate of fast-inactivation of the mutant channel with no change in other fast-gated properties. At the cellular level, this mutation would increase the persistent sodium current and is predicted to generate neuronal hyperexcitability.



**Genetics studies of neuronal nicotinic acetylcholine receptors in patients with megacystis and intestinal pseudo-obstruction.** *E. Lev-Lehman, M. Lising, D. Bercovich, W. Xu, A.L. Beaudet.* Molecular & Human Genetics, Baylor College Medicine, Houston, TX.

We are conducting a mutational analysis in patients with the rare autosomal recessive disorder megacystis, microcolon, intestinal hypoperistalsis syndrome (MMIHS) and the related syndrome of chronic intestinal pseudo-obstruction (CIP). Both of these disorders are characterized by abdominal distension, intestinal hypoperistalsis, intolerance to enteral feeding and in MMIHS also megacystis and abnormal voiding pattern. The phenotypic findings in these patients resemble the phenotype observed in mice lacking the  $\alpha 3$  subunit of the neuronal nicotinic acetylcholine receptor (nAChR) and in part, the phenotype observed in mice lacking the  $\beta 4$  subunit of the nAChR. This led to the hypothesis that mutations in the  $\alpha 3$  and/or the  $\beta 4$  subunits of the neuronal nAChR genes (*CHRNA3* and *CHRNA4*, respectively) might cause MMIHS or the related syndrome of CIP. We are therefore conducting a mutational analysis in these genes in patients with MMIHS or CIP. Both genes are mapped on chromosome 15q24.2-q25.1 in a cluster with the *CHRNA5* gene. In one patient, we found a possible splice mutation in the *CHRNA3* gene IVS2-14A@G which might cause exon skipping. In the *CHRNA4* we found a missense mutation of uncertain significance (Arg136Gln) in another patient. In addition, we identified several unprocessed partial pseudogenes of the *CHRNA4* gene on chromosome 15. The presence of a cluster of homologous genes and pseudogenes raises the possibility of complex genomic rearrangements that may be difficult to decipher. At this time, none of the changes is proven to be pathological, and we are interested in continuing the studies with additional patients affected with MMIHS or CIP.

**Expression Profiling of Menkes Disease Brain.** *P.-C. Liu<sup>1</sup>, Y.-W. Chen<sup>2</sup>, E.P. Hoffman<sup>2</sup>, S.G. Kaler<sup>1</sup>*. 1) Natl Inst Neurologic Disorders & Stroke, NIH; 2) Resesearch Ctr for Genetic Medicine, Children's National Med Ctr, Washington, DC.

Menkes disease (MD) is an X-linked recessive neurodegenerative disorder caused by mutations in a copper transporting P-type ATPase that normally delivers copper to the central nervous system. The precise reasons underlying the neurodegeneration in affected individuals are poorly understood. We hypothesized that gene expression changes in brain from patients with lethal mutations would suggest pathophysiological cascades associated with copper deficiency in the developing brain. To test this hypothesis, oligonucleotide probes for approximately 12,000 human gene sequences arrayed on Affymetrix Human Genome U95 GeneChips were used for expression profiling of fluorescently labeled primary cRNAs from postmortem brain of two well characterized patients who died at 5 and 7 months of age, respectively, despite very early (*in utero* in one case) copper histidine treatment. In both patients, the brains were harvested within 6 hours postmortem and were stored at -80 degrees C. As control tissue, we obtained similar high quality brain specimens from a Brain and Tissue Bank (University of Maryland). The control specimens selected (#463, frontal cortex and cerebellum; #569 frontal cortex) were obtained from normal infants who died accidentally, and who matched our patients for age, gender and race. Data analysis (completed by Affymetrix Microarray Suite v4.0 and Silicon Genetics GeneSpring) showed 60 genes significantly up-regulated ( $p < 0.001$ ) and 330 genes down-regulated ( $p < 0.001$ ) in the MD brains compared to the normal controls. Many of the 330 down-regulated genes were involved in signaling pathways, synaptic function, mitochondrial function, protein modification and metabolism. The majority of known up-regulated genes were involved in compensatory responses, including growth factors, anti-apoptosis, immune response and stress response genes. These findings provide considerable insight regarding the pathogenetic basis of neurodegeneration in MD patients with poor response to very early treatment with subcutaneous copper histidine injections.

**Two antioxidant proteins (TSA and PAG) interact in vivo with *Drosophila Psn* and may be candidates for Alzheimers disease.** *M.F. Wangler<sup>1</sup>, L.T. Reiter<sup>1</sup>, J.-J. Wu<sup>2</sup>, E. Bier<sup>1</sup>.* 1) Biology, UCSD, La Jolla, CA; 2) Pediatrics and Molecular Biology, Washington University School of Medicine, St. Louis, MO.

Presenilin 1 (*PSEN1*) is a transmembrane protein which when mutated in humans can result in the most prevalent form of familial early-onset Alzheimer's disease (FAD). Studies have shown that FAD mutations in *PSEN1* can lead to over-production of a neurotoxic form of beta-amyloid found in senile plaques. Although the cellular function of *PSEN1* is largely unknown, *PSEN1* has been implicated in diverse cellular activities including activation of the Notch and Wnt developmental signaling pathways, calcium homeostasis, and apoptosis. Evidence suggests that presenilins play a role in antioxidant biology, although no direct interactions with antioxidant proteins have been reported. We identified protein-protein interactions between human *PSEN1* and two antioxidant proteins, TSA and PAG, through a yeast two-hybrid screen using *PSEN1* as bait. We tested for *in vivo* interactions between these proteins by misexpressing them in *Drosophila* with fly *Psn* using the GAL4-UAS transactivation system. When TSA and PAG are coexpressed with *Drosophila Psn* in the wing and brain of flies we observed synergistic effects demonstrating that these proteins function in concert *in vivo* as well as *in vitro*. TSA and PAG alone generate no phenotype when misexpressed individually. As others have shown, *Psn* misexpression gives a weakly penetrant phenotype that appears to compromise Notch signaling and results in apoptosis. When PAG and or TSA are coexpressed with *Psn*, we observe a strong enhancement of this phenotype indicating that these proteins function in a concerted fashion. We also analyzed expression of Notch target genes and found that coexpression of PAG, TSA, and *Psn* interferes with the Notch mediated activation of these genes. These studies were extended to neural tissue by using brain specific GAL4 drivers to analyze interactions in the brain. Our analyses suggest that TSA and PAG play an important role in regulating apoptosis and Notch signaling through its interaction with *Psn*. We suggest that human genes like TSA and PAG should be considered as candidate genes for yet undetermined FAD loci.

**Antemortem diagnosis of Alexander disease in a Japanese patient by molecular genetic analysis.** *N. Shiroma*<sup>1,2</sup>, *N. Kanazawa*<sup>1</sup>, *M. Izumi*<sup>2</sup>, *K. Sugai*<sup>2</sup>, *M. Fukumizu*<sup>2</sup>, *M. Sasaki*<sup>2</sup>, *S. Hanaoka*<sup>2</sup>, *M. Kaga*<sup>2</sup>, *S. Tsujino*<sup>1</sup>. 1) Inherited Metabolic Disease, National Inst Neuroscience, Kodaira, Tokyo, Tokyo, Japan; 2) Neuropediatrics, Musashi Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan.

Alexander disease is a rare type of leukodystrophy characterized clinically by macrocephaly, psychomotor retardation, spasticity and seizures, and histopathologically by the presence of numerous Rosenthal fibers (RF), cytoplasmic inclusion bodies in astrocytes. Recently, mutations in the gene encoding glial fibrillary acidic protein (GFAP) were identified in patients with Alexander disease. We sequenced the GFAP gene of a Japanese girl who presented with typical symptoms of Alexander disease but in whom the diagnosis was not proven by histopathology. We identified a missense mutation, R239C, which is identical to the mutation previously reported to be most frequent. As was the case in previously-described patients, our patient was also heterozygous for the de novo mutation. Interestingly, in spite of the fact that this is a de novo mutation, R239C was found to be common in different ethnic groups, implying that the site is a "hot spot" for mutagenesis. Molecular genetic analysis now makes the antemortem diagnosis of Alexander disease possible.

**SPG4 (spastin) mutation screening in hereditary spastic paraparesis.** *C. Proukakis<sup>1,2</sup>, C. Comiskey<sup>1</sup>, E. Reid<sup>3</sup>, P. Wilkinson<sup>2</sup>, D. Rubinsztein<sup>3</sup>, M.A. Patton<sup>1</sup>, T.T. Warner<sup>2</sup>, A.H. Crosby<sup>1</sup>.* 1) Department of Medical Genetics, St George's Hospital Medical School, London SW17 0RE, UK; 2) Department of Clinical Neurosciences, Royal Free Campus, Royal Free and University College Medical School, London NW3 2PF, UK; 3) Department of Medical Genetics, Cambridge University, Cambridge CB2 2XY, UK.

Hereditary spastic paraparesis (HSP) is a clinically and genetically heterogeneous condition. Inheritance is commonly autosomal dominant (AD), although recessive and X-linked forms exist. It has been estimated that up to 40% of AD pedigrees show linkage to the SPG4 locus at 2p21-22 which encodes spastin, an AAA protein (ATPase associated with diverse cellular activities).

Over 60 SPG4 mutations have been reported to date, with most occurring only in one family. These are scattered across most of the gene's 17 exons and include missense, nonsense and splicing mutations as well as insertions and deletions. SPG4 mutations are not fully penetrant, there is marked intrafamilial variability and there is one report of a mutation in a sporadic case apparently acting as recessive. The above problems have made large-scale diagnostic testing problematic.

In order to determine the frequency of SPG4 mutations in a cohort of patients diagnosed as HSP, we have obtained genomic DNA from over 90 unrelated patients. Most of these had a family history, and in some cases were members of pedigrees displaying clear AD inheritance.

All 17 exons of SPG4 were amplified by PCR (exon 1 was amplified as 3 separate fragments) and subjected to single strand conformation polymorphism (SSCP) analysis. All samples with apparent band shifts were sequenced on an ABI377 automated sequencer. Confirmation by subcloning (using a TOPO cloning kit) and sequencing was undertaken where appropriate.

We have detected a number of spastin mutations amongst this large collection of patients. A comparison of spastin mutation frequency is offered between familial and apparently sporadic cases.

**Diagnosis of spinal muscular atrophy for deletions of SMN: is it possible?** *V. Sokolnik, R. Khmel, G. Tsukerman.*  
Institute for Hereditary Diseases, MINSK, Belarus.

We have performed SSCP analysis of the SMN gene exons 7,8 and electrophoretic study of the NAIP gene exons 5,13 in 37 Belarus families (38 probands, submitted with a presumptive clinical diagnosis of SMA, and 67 SMA-asymptomatic family members). The control consisted of 40 dried blood spots from newborns. Of 38 patients with SMA, 31 have deletion changes of SMNt gene. The NAIP gene exon 5 was deleted in severe affected patients (9 of 23 presumptive SMA type I cases). All patients, who lacked the NAIP gene exon 5, also had deletion mutations of SMNt. Deletions of SMNt exon 7 alone were in both severe affected patients and milder ones. We observed three unaffected parents deleted for exon 5 of NAIP gene. In one of these families SMA-unaffected mother and SMA type I patient showed loss of band corresponding to SMNt exon 7. In another two cases child with SMA type I exhibited a deletion of SMNt exons 7, 8 and NAIP gene exon 5. We believe that two SMA-asymptomatic parents had deletion of exons 7 and 8 in the centromeric gene (SMNc). In one of these families the proband was deleted for both exons 7 and 8 of SMNt, in another one - for SMNt exon 7 only. In addition, mutation changes, which looked like a deletion of centromeric exons 7 and 8, were found in one (boy) of two patients with clinical diagnosis of SMA type I. In this family the mother had identical SSCP patterns, except that PCR product of exon 7 showed also extra bands with altered mobility. The father and second child (girl) retained at least one copy of both genes. Finally, absence of bands corresponding to exon 7 and 8 of SMNc have been demonstrated in the father of a patient with diagnosis of SMA, which subsequently was changed on muscular dystrophy. Control newborns showed neither the deletion mutations in SMNt nor the absence of NAIP exon 5. Deletions in SMNc have been demonstrated by SSCP in 5 of 40 controls and were represented by the deletions of centromeric exon 7 bands or deletions of both centromeric exon 7 bands and centromeric exon 8 band. Deletion analysis of control group and revision of clinical features of SMA families now are in progress.

**Mutation analysis of ATP7B gene in Russian Wilsons Disease patients.** *M.I. Shadrina<sup>1</sup>, A.O. Davydova<sup>1</sup>, P.A. Slominsky<sup>1</sup>, S.A. Limborska<sup>1</sup>, A.V. Karabanov<sup>2</sup>, I.A. Ivanova-Smolencskaya<sup>2</sup>.* 1) Dept Human Genetics, Inst Molecular Genetics, Moscow, Russia; 2) Institute of Neurology, Russian Academy of Medical Science, Moscow, Russia.

Wilson disease (WD) is an autosomal recessive disorder characterised by toxic accumulation of copper in liver and subsequently in brain and other organs. The world-wide incidence of this disease is about 30 per million, with the gene frequency of 0.56% and carrier frequency of 1 per 90. The WD ATP7B was mapped to chromosome 13q14.3. This gene is expressed most abundantly in liver and encodes a copper-transporting P-type ATPase. A large number of mutations of ATP7B gene have been identified. This study aimed to detect disease-causing mutations of ATP7B gene in Russian population.. We analyzed 33 WD patients by SSCP analysis, sequencing and rapid detection method for the most frequent in Russia His1069Gln mutation. We found that one patient was homozygous and 11 patients were heterozygous for the His1069Gln mutation, which is the most common mutation. Also we detected two mutations in exon 8 of ATP7B gene, one of these was a novel missense mutation (Trp712Cys). This mutation destroys restriction site for Rsa I. The same site was destroyed by known mutation Tyr713Cys. In two patients Gly710Ser missense mutation was found. Overall frequency of exon 8 mutations in non-His1069Gln alleles is 5,7%.

**Two spastin isoforms are developmentally regulated in fetal and adult human brain.** *E. Pegoraro*<sup>1</sup>, *A.M. Molon*<sup>1</sup>, *A. Fassina*<sup>2</sup>, *P. Magalhaes*<sup>3</sup>, *C. Angelini*<sup>1</sup>. 1) Neurological/Psychiatric Sci, Univ Padova, Padova, Italy; 2) Oncological and Surgical Sciences, University of Padova, Italy; 3) Department of Biomedical Sciences, University of Padova, Italy.

Spastin is a newly described protein belonging to the AAA protein family (ATPase Associated with different cellular Activities). Homology with the 26S proteasome of *Saccharomyces cerevisiae* suggests that spastin may be involved in transcriptional regulation of DNA; its functional role in the central nervous system, however, remains largely unknown. Two isoforms of spastin are generated by alternative splicing of exon 4: full length spastin (FL-SPG4), that retains exon 4, and exon 4 alternatively spliced spastin (AS-SPG4) that lacks 32 amino acid residues in a region of the protein showing, through blast searches, homology to a microtubule associated protein (MAP2). We examined the expression pattern of the two isoforms in post-mortem fetal and adult human brains by quantitative multiplex fluorescent RT-PCR (QMF-RT/PCR) through co-amplification of cDNAs corresponding to survival motor neuron (SMN), and spastin gene (SPG4). In the fetal central nervous system including corpus callosum and spinal cord both the FL-SPG4 and the AS-SPG4 were highly expressed with a clear predominance of the FL-SPG4. In adult, FL-SPG4 mRNA was mainly expressed in the cerebral cortex and cerebellum, whereas AS-SPG4 mRNA was mainly expressed in the corpus callosum and spinal cord where FL-SPG4 was barely detectable. Down-regulation of SPG4 in the corpus callosum and spinal cord started during early postnatal stages. Therefore, splicing of exon 4 undergoes distinct temporal and spatial regulation in the brain. The role of the AS-SPG4 is still unknown, however the homology shown by FL-SPG4 with MAP2 is lost when exon 4 is skipped suggesting a possible role of spastin in microtubule assembly.



**GBX2: a candidate gene for midline cerebellar defects.** G. Zanni<sup>1,2</sup>, F. Savini<sup>3</sup>, FM. Santorelli<sup>1</sup>, G. Neri<sup>2</sup>, A. Giannotti<sup>1</sup>, E. Bertini<sup>1</sup>. 1) Molecular Medicine, Bambin Gesù Children's Hospital Rome, Italy; 2) Medical Genetics, Catholic University, Rome; 3) Medical Genetics, University La Sapienza, Rome, Italy.

Genes regulating early specification and development of the cerebellum are attractive candidates to be investigated in malformation syndromes characterized by midline cerebellar defects. Joubert syndrome (JS) is a rare autosomal recessive disorder characterized by agenesis/dysgenesis of the cerebellar vermis with accompanying brainstem malformations and other developmental defects including ocular, renal, hepatic and limb abnormalities. This condition is clinically and genetically heterogeneous. One locus was identified on chromosome 9q34 but so far no responsible gene was found. Dandy Walker Malformation (DWM) is characterized by vermis agenesis/hypoplasia and cystic dilation of the 4th ventricle and is an associated symptom in many malformation syndromes. GBX2 (gastrulation and brain specific 2) gene, is a vertebrate homeobox related to *Drosophila unplugged* gene, which plays an essential role in the establishment of the isthmus organizer (IsO), a source of positional information for the development of midbrain and anterior hindbrain structures, at the mes-metencephalic junction of the developing embryo. It has been postulated that early abnormalities at the mesencephalic side of the isthmus could contribute to the pathogenesis of midline cerebellar defects. GBX2 null mutant mouse lacks normal rostral hindbrain and presents IsO abnormalities, while in GBX2 hypomorphic mouse mutant, the cerebellar vermis fails to develop. We have analyzed the GBX2 gene in 14 patients with cerebellar vermis abnormalities, 5 of them fulfilling the diagnostic criteria for JS and 5 with syndromal DWM. PCR was carried out using intronic and exonic primers for the two exons of GBX2. Mutation analysis was performed by direct sequencing using Big Dye Terminator Cycle sequencing kit. No differences were noted in the coding sequence of GBX2 between normal and affected individuals. However, the implication of GBX2 (as of other IsO genes: FGF8, WNT1, PAX2, EN 1-2 previously tested in Joubert patients) in the pathogenesis of cerebellar defects cannot be definitively ruled out.

**Caspr1/Paranodin/Neurexin IV is most likely not a common disease-causing gene for inherited peripheral neuropathies.** *V. Timmerman<sup>1</sup>, K. Venken<sup>1</sup>, J. Meuleman<sup>1</sup>, J. Irobi<sup>1</sup>, C. Ceuterick<sup>2</sup>, R. Martini<sup>3</sup>, P. De Jonghe<sup>1,4</sup>.* 1) Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), University of Antwerp (UIA), Antwerpen, Belgium; 2) Born-Bunge Foundation (BBS), University of Antwerp (UIA), Antwerpen, Belgium; 3) Department of Neurology, Section of Developmental Neurobiology, University of Wurzburg, Wurzburg, Germany; 4) Division of Neurology, University Hospital Antwerpen (UZA), Antwerpen, Belgium.

Contactin associated protein 1 (Caspr1/Paranodin/Neurexin IV) is an axonal transmembrane molecule mainly localised at the paranodal junction. Since molecular alterations in septate-like junctions at the paranodes might have important consequences for the function of the nerve fiber, we considered that Caspr1 could be involved in the pathogenesis of inherited peripheral neuropathies. We screened a BAC library to isolate clones containing the complete coding region of Caspr1. Full genomic characterisation of human Caspr1 (1384 AA, ORF of 4155 nucleotides) resulted in the determination of 24 coding exons. Metaphase FISH was used to localise the Caspr1 gene on chromosome 17q21. Using DHPLC and direct DNA sequencing we found 7 different sequence variations in 63 unrelated patients afflicted with distinct inherited peripheral neuropathies. Four sequence variations (c.151C>A, c.1861C>A, c.2340C>A and c.3588G>A) resulted in silent mutations and 1 variation (c.1577G>A) resulted in the amino acid change R526Q. None of these heterozygous or homozygous (c.151C>A) mutations co-segregate with the disease phenotype in the corresponding families. Furthermore, two splice-site mutations were found at IVS7-5C>G(1045-5C>G) and IVS10+5C>T(1628+5C>T). Both splice-site mutations were not localised in the most conserved nucleotides of the splice-donor (GT) and splice-acceptor (AG) sites, and did also not co-segregate within the patients families. We therefore conclude that Caspr1 is most likely not involved in the molecular pathology of inherited peripheral neuropathies in our patient cohort.

**Mutation screening of SPTLC1 in patients with Hereditary sensory neuropathy type I .** *K. Verhoeven<sup>1</sup>, E. De Vriendt<sup>1</sup>, V. Van Gerwen<sup>1</sup>, M. Auer-Grumbach<sup>2</sup>, A. Pou-Serradell<sup>3</sup>, P. de Jonghe<sup>1,4</sup>, V. Timmerman<sup>1</sup>.* 1) Flanders Interuniversity institute for Biotechnology (VIB), Born-Bunge foundation (BBS), University of Antwerp, Antwerpen, Belgium; 2) Department of Neurology, Karl-Franzens University, Auenbruggerplatz 22, A-8036 Graz, Austria; 3) Department of Neurology, University of Barcelona, Barcelona, Spain; 4) Division of Neurology, University Hospital Antwerpen (UZA), Antwerpen, Belgium.

Hereditary Sensory Neuropathy type I (HSNI) is a dominantly inherited degenerative disorder of peripheral sensory neurons. Symptoms of HSNI start in the second to third decade with loss of pain sensation followed by distal muscle wasting and weakness. Loss of pain sensation leads to chronic skin ulcers, requiring amputation. HSNI was genetically linked to chromosome 9q22.1-q22.3. Mutations in the serine palmitoyltransferase, long chain base subunit 1 gene (SPTLC1) were identified as underlying HSNI. Using direct DNA sequencing we screened a panel of 19 unrelated patients afflicted with HSNI. In a small Belgian family, two twin sisters carried a missense mutation (c.387G>A) which resulted in a G387A amino acid substitution. Furthermore, one splice-site mutation was found at IVS8-6 A>G (781-6A>G) in a small Spanish family with two patients. Both mutations were not found in 50 control patients. Interestingly, up until now all known mutations in the SPTLC1 gene were found in exon 5 and 6, while we found two novel mutations located in exon 13 and in the splice acceptor site of exon 9.

**Altered recruitment of 26S proteasome subunits and chaperones into neuronal intranuclear inclusions in SCA3.**

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Spinocerebellar Ataxia Type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a polyglutamine tract in the ataxin-3 protein. A neuropathological hallmark of SCA3 are intracellular aggregates forming neuronal intranuclear inclusions (NII). To characterize cellular responses to nuclear aggregates in more detail, we analysed nuclear inclusions in human postmortem brain of patients affected by SCA3 and controls immunohistochemically using a panel of antibodies directed against chaperones and proteasome subunits. About 20% of the nuclear inclusions in pontine neurons stained positively for the chaperone Hsp90a, whereas no staining by antibodies to Hsp27, Hsp60, and Hsp/Hsc70 was observed. Most nuclear inclusions in SCA3 are ubiquitin-positive (75%) suggesting degradation by ubiquitin-dependent proteasome pathways. Surprisingly only a minority of the inclusions were immunopositive with antibodies directed against subunits of the 20S proteolytic core of proteasomes, whereas most inclusions were stained by antibodies directed against the 19S regulatory subunits thought to recognize, bind and unfold ubiquitinated proteins. In addition, most NII were immunoreactive with 11S subunits antibodies. A comparison with normal control brains indicates more intense cytoplasmatic staining and less intense nuclear signals in SCA3 neurons suggesting a redistribution of proteasomes. The high proportion of NII stained with proteasome subunits 11S and 19S stands in marked contrast to the small percentage of NII immunostained with the 20S antibodies used. These results suggest that most neurons containing NII recruit proteasome subunits to NII possible in an attempt to refold mutant ataxin-3 and to dissolve nuclear aggregates.

**Characterization of the interaction between neurofibromin and members of the syndecan family.** *M. Volta*<sup>1</sup>, *Y. Hsueh*<sup>2</sup>, *M. Sheng*<sup>3</sup>, *R.G. Roberts*<sup>1</sup>. 1) Div. of Med. & Mol. Genet., Guy's Hospital, London, UK; 2) Inst. of Mol. Biol. Academia Sinica, Taipei, Taiwan, China; 3) Howard Hughes Medical Inst. & Dept. of Neurobiology, Massachusetts General Hospital, Boston, Massachusetts, USA.

Neurofibromatosis type 1 (NF1) is a common dominantly inherited disorder with symptoms which can include life-threatening benign and malignant nervous system tumours, skeletal abnormalities and learning disabilities. Although the identification of a small central region (GTPase activating domain) of neurofibromin, which acts to down-regulate members of the Ras family of small GTPase, could partially explain the tumor development in NF1 patients, the more complex events, which lead to the expression of the other phenotypic traits of this disease are still unknown.

We have recently identified an interaction between two distinct regions of neurofibromin and members of syndecan protein family of heparan sulphate proteoglycans (HSPGs). As syndecans are implicated in a range of regulatory aspects of the interactions between cells and their environment they represent particularly interesting candidates for functional partners of neurofibromin.

We subsequently set out to characterize this interaction in more detail by introducing a series of point mutations into the cytoplasmic domain of syndecan-2 and tested them in yeast two-hybrid experiments. Surprisingly, most of these mutations affected the binding of the two regions of neurofibromin and that of CASK (another syndecan-binding protein) in an almost identical way. In an attempt to explain this, we assessed the effect of these mutations on the interaction of syndecan-2 with itself (a dimerization interaction). This yielded the same pattern of results as neurofibromin and CASK. We interpret this as suggesting that syndecan dimerization is a prerequisite for interaction with neurofibromin and CASK. We were also able to identify residues which mediates specific interaction with neurofibromin.

These findings imply that syndecan genes could represent modifier loci for NF1 phenotype.

**The detection of hot spot mutations in exon three of MECP2 gene in Rett syndrom patients of Slavic origin.** R. Rosipal<sup>1</sup>, J. Hadac<sup>2</sup>, J. Zeman<sup>1</sup>, N. Misovicova<sup>3</sup>, S. Nevsimalova<sup>4</sup>, P. Martasek<sup>1</sup>. 1) Department of Pediatrics, Charles University, Prague, Czech Republic; 2) Thomayer Hospital, Prague, Czech Republic; 3) Martin Hospital, Martin, Slovak Republic; 4) Department of Neurology, Charles University, Prague, Czech Republic.

Mutations in X-linked MECP2 gene cause Rett syndrome - an X-linked dominant neurodevelopmental disorder affecting 1 in 10,000 to 15,000 females worldwide (1). MECP2 gene encodes for methyl-CpG-binding protein 2, which plays an important role in the regulation of gene expression (2). The spectrum of mutations in MECP2 gene is now known from numerous countries and ethnic groups and steps are being taken to determine the genotype/phenotype relationship in order to better understand the disease process (3,4). A systemic study in the Slavic population was initiated from which we present the results of 25 girls from the Czech and Slovak republics with established clinical diagnoses of Rett syndrome. There are 5 prevalent mutations in the methyl-CpG binding protein 2, that result in Rett syndrome. Four of them are detectable by restriction analysis. In this study we present the results of the search for 4 such prevalent mutations in the gene for methyl-CpG binding protein 2. Restriction analysis of PCR products of the methyl-CpG binding protein 2 gene revealed the presence of these 4 mutations [473 C/T (T158M) in 3 patients, 502 C/T (R168X) in 2 patients, 808 C/T (R270X) in 1 patient, and 906 C/T (R306C) in 2 patients] in 8 unrelated patients with Rett syndrome. The results of restriction analysis were confirmed by direct sequencing. This study of Rett syndrome in the Slavic population shows that 4 of the hot spot mutations in exon 3 of MECP 2 gene are present in 32% of the patients and dictates, therefore, the strategy in molecular diagnosis of Rett syndrome in this ethnic group. 1. Rett, A.: Wien Med. Wochenschr. 116, 1966, 723-726. 2. Amir, R.E., et al. : Nature Genet. 23, 1999, 185-188. 3. Xiang, F., et al.: J. Med. Genet. 37, 2000, 250-255. 4. Cheadle, J.P., et al.: Hum. Mol. Genet. 12, 1119-1129, 2000. Supported by Czech Granting Agency (GACR 301/01/P068 and 302/99/0648).

**Mutational analysis of Cx32, MPZ, PMP22 and NEFL genes in 32 Chinese families with Charcot-Marie-Tooth Disease.** *J. Xiao<sup>1,2</sup>, B. Tang<sup>1</sup>, J. Xia<sup>1</sup>, G. Xie<sup>1</sup>, W. Luo<sup>1</sup>.* 1) Dept Neurology, Xiangya Hospital, Hunan Medical University, Changsha, Hunan, China. 410008; 2) Dept Neurology, Neuroscience Ctr, Memphis, TN.

Objective: To study the molecular pathogenesis of Charcot-Marie-Tooth disease (CMT) in China. Methods: 81 patients of CMT from 32 families around China were studied both in clinic and genetics. Mutational analysis of Cx32, MPZ, PMP22 and NEFL genes were performed by PCR-RFLP, PCR-SSCP, PCR-DGGE and /or direct sequencing. Results: 8 (25%) of the CMT families had mutations in the Cx32, MPZ and PMP22 genes. Four point mutations were found in the second exon of the Cx32 gene. They are 188ACC®GCC (Thr®Ala) in a XD family, 15CGG®CAG (Arg®Gln) and 183CGC®CAC (Arg®His) in two XR families and 164CGG®CAG (Arg®Gln) in a sporadic patient. One point mutation was found in MPZ gene (124ACG®ATG Thr®Met) in a AD family. Three tandem CMT1A-REP repeat mutations of PMP22 gene were found in three AD families. However, There is no point mutation in these patients both in PMP22 and NEFL gene. Conclusion: The results showed that Chinese CMT patients share the same mutations in Cx32, MPZ and PMP22 genes in the world although there is a little difference in the mutation site and inheritance patterns. However, most of the families without mutations in these genes need further research.

**Assessment of Glypican 3 gene mutation in patients referred for overgrowth syndrome.** *V. Gaston<sup>1</sup>, Y. Le Bouc<sup>1</sup>, L. Burglen<sup>2</sup>, V. Soupre<sup>3</sup>, M.P. Vazquez<sup>3</sup>, C. Gicquel<sup>1</sup>.* 1) Lab. d'endocrinologie -INSERM U515, hopital Trousseau, AP-HP, Paris, France; 2) service de genetique medicale, hopital Trousseau, AP-HP, Paris, France; 3) service de chirurgie maxillo-faciale, hopital Trousseau, AP-HP, Paris, France.

Overgrowth syndromes (OGS), include the well-studied Beckwith-Wiedemann syndrome (BWS) and the Simpson-Golabi-Behmel syndrome (SGBS) which have overlapping phenotypes and are often difficult to distinguish on a clinical background. The most frequent, BWS results from variable mutations or epigenetic modifications of imprinted genes in the 11p15 chromosomal region. SGBS is an X-linked syndrome involving mutation in the glypican-3 gene (GPC3) which encodes a heparan sulfate proteoglycan. As there are a great phenotypic variability in SGBS with the possibility of mild forms, and an overlapping phenotype with BWS, the aim of this study was to evaluate the incidence of SGBS in patients referred for OGS. From 144 patients (including 8 familial cases) referred for OGS, fifty-nine (41 %) did not exhibit abnormality in the 11p15 region (abnormal demethylation of KvDMR1, 11p15 uniparental disomy, isolated hypermethylation of the H19 gene or germline CDKN1C mutation). Among them, the 31 male patients were screened for GPC3 gene mutation. The entire coding region of GPC3 including intron-exon boundaries was analyzed by PCR and SSCP and all PCR products with aberrant SSCP migration patterns were directly sequenced. Two patients showed a point mutation that predict a loss-of-function of the GPC3 protein: a frameshift CG231T mutation and a missense mutation, V479M, changing a conserved aminoacid in a sporadic case. One patient exhibited a deletion involving exons 7 and 8 of the GPC3 gene. Two other unrelated patients showed the same 4 bp microdeletion in the 3' UTR which was not detected in the control population (174 X chromosomes analyzed). Two missense mutations (Q521H and G606R) were also found in two other patients directly referred with a clinical diagnosis of SGBS. Although mutations in the GPC3 gene are uncommon in the overall population of OGS patients, GPC3 analysis is appropriate in male patients without abnormality in the 11p15 region and may contribute to the genetic counseling.



**Karyotypic abnormality in a child whose phenotype overlaps Beckwith-Wiedemann syndrome defines a new overgrowth syndrome locus in 18qter.** *F. Lirussi<sup>1</sup>, D. Sanlaville<sup>2</sup>, V. Gaston<sup>1</sup>, C. Houdayer<sup>1</sup>, V. Soupre<sup>1</sup>, R.*

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Beckwith-Wiedemann syndrome (BWS) is a tumor-predisposing condition with cardinal features of exomphalos, macroglossia, and gigantism in the neonate. BWS has been related to genetic imbalance in complex 11p15.5-linked region, which contains several imprinted genes. Other genetic loci may be involved in overgrowth syndromes in overlap with BWS. In particular, deletion 18q22.1 was shown in an infant with findings of macroglossia, umbilical hernia, neonatal hypoglycemia, and postnatal overgrowth. We report on a boy, term-born by C-section in a state of apparent death, who had findings of neonatal hypoglycemia, bilateral inguinal hernia, and other severe developmental problems. His tongue became progressively enlarged, causing major prognathism and hindering feeding. Adenoidomegaly caused airway to obstruct. There was a complex de novo chromosomal unbalance resulting from the presence, in all lymphocytes analyzed, of an abnormal monocentric chromosome 18 with partial duplication of 18q, and from a mosaic (50%), also exclusively 18-derived, small monocentric marker without identifiable subtelomeric specific sequences by FISH. The marker and der(18) were both characterized using a whole-chromosome paint probe. FISH using probe TEL 18q (D18S1390) elicited a signal on the normal 18 only demonstrating the 18qter deletion on the der(18). Molecular analysis for *IGFII* ruled out uniparental disomy in both leucocyte and tongue (glossectomy-derived) DNAs, and showed normal, monoallelic (paternal), expression from tongue RNA. Methylation of *H19* was restricted to the paternal allele whereas *KCNQ1OT1/LIT1* was methylated on the maternal allele only. This observation supports the existence of a genetic locus in the distal end of chromosome 18 whose haplo-insufficiency could entail a phenotype in overlap with BWS without visible imbalance in the 11p15.5 region.

**Molecular mechanism of disease in Carney Complex patients with mutations in *PRKARIA*.** *L.S. Kirschner<sup>1</sup>, F. Sandrini<sup>1</sup>, S.M. Lenherr<sup>1</sup>, J.A. Carney<sup>2</sup>, C.A. Stratakis<sup>1</sup>.* 1) Unit on Genetics and Endocrinology, DEB, NICHD, Bethesda, MD; 2) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Carney Complex (CNC) is a multiple endocrine neoplasia syndrome characterized by tumors of the adrenal cortex, anterior pituitary, thyroid, and gonads. Non-endocrine manifestations of this disease include spotty skin pigmentation, myxomas, and pigmented schwannomas. Genetically, this autosomal dominant disease has been mapped to 2p16 and 17q22-24. We have identified 68 kindreds with CNC from the NIH and Mayo Clinic, and we have used this cohort to identify mutations of *PRKARIA* in families mapping to 17q. Mutations in this gene, which codes for the Type 1A regulatory subunit of the cAMP-dependent protein kinase (PKA), have been detected in 41.2% of our CNC kindreds (28/68).

At the molecular level, CNC-causing mutations in *PRKARIA* were spread throughout the coding region of the gene. The majority of these mutations (22/28) were nonsense or frameshift mutations, leading to a premature termination codon. There were 5 splice site mutations and one mutation which abolished the initiator ATG of the protein. Analysis of the *PRKARIA* protein from cell lines from CNC patients with mutations showed that the predicted truncated proteins are not produced within the cells. Similarly, mRNA levels for the truncating transcripts are markedly reduced compared to the wild-type. Analysis of this phenomenon has demonstrated that these mutant mRNAs are rapidly degraded in the cells through the mechanism of nonsense-mediated mRNA decay, thus leading to selective suppression of the mutant protein.

At the functional level, PKA activity in CNC tumor cells is hyperresponsive to cAMP, suggesting an increase in cAMP-mediated signalling in these cells. The same phenomenon is observable in EBV-transformed lymphocytes from patients with CNC, indicating these patients may have more global abnormalities in cAMP-dependent pathways. Similar findings are observed in CNC patients lacking *PRKARIA* mutations, suggesting that the second CNC gene is also involved in the PKA signalling pathway.

**Differential Gene Expression in Monocytes from Probands with Elevated Lipoprotein (a).** *C. Buechler<sup>1</sup>, H. Ullrich<sup>1</sup>, M. Ritter<sup>1</sup>, K.J. Lackner<sup>1</sup>, S.O. Friedrich<sup>1</sup>, G.M. Kostner<sup>2</sup>, G. Schmitz<sup>1</sup>.* 1) Clin Chem/Lab Med, Univ Regensburg, Regensburg, Germany; 2) Med Biochem, Univ Graz, Graz, Austria.

Elevated plasma Lp(a) and cardiac events show a modest but significant association in various clinical studies. However, the influence of high Lp(a) on the gene expression in blood monocytes as a major cell involved in atherogenesis is poorly described. To identify genes influenced by elevated serum Lp(a) we analysed the gene expression on a cDNA microarray comparing monocytes from a patient with isolated Lp(a) hyperlipidemia and coronary heart disease with monocytes from a healthy blood donor with low Lp(a). Using this approach numerous genes were found differentially expressed in patient versus control monocytes. Verification of these candidates by Northern blot analysis or semiquantitative PCR in monocytes from additional patients with Lp(a) hyperlipidemia and healthy blood donors with elevated Lp(a) confirmed a significant induction of PAI-2 mRNA in monocytes from male but not from female individuals with high Lp(a) indicating that this observation is gender specific. This led also to increased intracellular and secreted PAI-2 protein in monocytes from male probands with Lp(a) hyperlipidemia. PAI-1 and LAL mRNA was found suppressed only in the patients monocytes and not in healthy probands with high Lp(a) levels. Purified Lp(a) induced PAI-2 mRNA and protein and reduced PAI-1 and LAL expression in monocytes isolated from various controls. The finding that PAI-2 is elevated in monocytes from male patients with isolated Lp(a) hyperlipidemia and male healthy probands with high Lp(a) and that purified Lp(a) upregulates PAI-2 in control monocytes in-vitro indicate a direct, but gender specific effect of Lp(a) for the induction of PAI-2 expression.

**Homozygous familial hypercholesterolaemia: Multiple founder mutations underlie phenotypic variation in the South African population.** *M. Callis<sup>1</sup>, S. Jansen<sup>1</sup>, R. Thiart<sup>2</sup>, M.J. Kotze<sup>2</sup>*. 1) Department of Human Genetics, University of the Free State, Bloemfontein, Free State, South Africa; 2) MRC Cape Heart Group, Division of Human Genetics, University of Stellenbosch, Tygerberg, South Africa.

**Objective.** Predominance of three low density lipoprotein receptor (LDLR) gene mutations in South African Afrikaner patients with familial hypercholesterolaemia (FH) significantly enhances the diagnostic prospects of the disease in this population. The degree of genetic heterogeneity in Afrikaner FH homozygotes was studied in relation to the phenotypic expression of the disease, in order to determine whether additional mutations should be included for routine DNA diagnosis, and to assess whether mutation status may provide useful parameters of disease progression in homozygous FH.

**Methods.** Polymerase chain reaction (PCR)-based methods were used to screen FH homozygotes without (or with only one copy of) the three founder-related mutations, D154N, D206E and V408M, for disease-related LDLR gene mutations. Afrikaner FH homozygotes with these mutations known to cause a receptor-defective or -negative phenotype, were grouped according to genotype for comparative analysis.

**Results.** Mutations W66G, D200G (heterozygous state) and S285L (homozygous state) were identified in three of the four Afrikaner FH homozygotes subjected to mutation analysis. Significantly higher mean total- ( $P < 0.008$ ) and LDL-cholesterol ( $P < 0.015$ ) levels were demonstrated in 24 FH homozygotes with at least one copy of the receptor-negative mutation V408M, compared with 23 patients with receptor-defective LDLR gene mutations D154N and/or D206E.

**Conclusions.** Mutation analysis provides useful parameters on which to base predictions of clinical progression of FH. Inclusion of all relevant mutations in routine DNA screening would therefore facilitate an improved diagnostic service for FH in the genetically homogeneous Afrikaner population.

**Lamin C mutation associated with variable expression of dilated cardiomyopathy and conduction-system disease (D-CM) and limb girdle muscular dystrophy type 1B (LGMD1B) in four generations of a large kindred. S.**

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LMNA mutations have been described in four distinct medical conditions including dilated cardiomyopathy with conduction-system defects (D-CM), autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD-AD), limb girdle muscular dystrophy type 1B (LGMD1B), and familial partial lipodystrophy (FPLD). Lamin A and C are nuclear proteins that are encoded by the LMNA gene and differ from each other only through alternative splicing of exon 10. We report here a unique family with a novel mutation, R541S, in exon 10 of the lamin C coding region of LMNA. There is variable expression of D-CM and LGMD1B in affected family members. Three affected individuals had severe, progressive D-CM resulting in sudden cardiac death. A 13 year old male with severe D-CM is now the youngest reported child with a LMNA mutation to develop D-CM and to require a cardiac transplant. Two members presented with a myopathy, subsequently discovered to be LGMD1B, and had less severe D-CM. One affected individual with mild LGMD1B had delayed ambulation as a child. Several asymptomatic individuals have cardiac conduction defects. Many affecteds have muscle pain and most have had an elevated CPK. Also noted is a distinct physical phenotype in females with a decrease in adipose tissue of the extremities, but not associated with the biochemical features of insulin resistance as seen in FPLD. This family illustrates that a single LMNA mutation may lead to the expression of several distinct phenotypes and, in light of these findings, it is suggested that individuals with LMNA mutations should be investigated for features of all of the above named conditions. In particular, it should be recognized that alterations in the lamin C sequence can lead to the development of severe D-CM and children with these mutations may require a heart transplant at a young age.

**Functional analysis of lamin A/C mutations leading to dilated cardiomyopathy, Emery-Dreifuss muscular dystrophy and partial lipodystrophy.** *G.L. Brodsky*<sup>1,2</sup>, *A.C. Moss*<sup>2</sup>, *L. Mestroni*<sup>1,2</sup>, *M.R.G. Taylor*<sup>1,2</sup>. 1) Dept Cardiology/Medicine, Univ Colorado Health Sci Ctr, Denver, CO; 2) University of Colorado Cardiovascular Institute, Aurora, CO.

Lamin A/C gene mutations have been identified in four different human diseases including Emery Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy (LGMD), dilated cardiomyopathy (DCM) and partial lipodystrophy (PLD). The lamin A/C gene encodes two proteins which are the primary components of the nuclear lamina; a proteinaceous meshwork lining the nucleoplasmic face of the inner nuclear membrane. Lamins have also been shown to form intranuclear filaments. The nuclear lamina has been implicated in nuclear structural integrity, chromatin organization, cell cycle regulation and transcriptional unit organization.

As a first step in elucidating the molecular pathway by which lamin A/C mutations lead to human disease, we have examined the expression and localization of disease causing mutant lamin A proteins in mammalian cell culture. Single nucleotide substitutions responsible for EDMD, DCM and PLD were introduced into a wild type lamin A cDNA and transfected into mouse F9 cells. The expression and sub-cellular localization of the mutant lamin A proteins were determined by indirect immunofluorescence deconvoluting confocal microscopy.

All of the mutant lamin A proteins examined were found to be expressed and localized to the cell nucleus. The mutant proteins responsible for EDMD and DCM produced a wild type pattern of distribution, localizing to the nuclear lamina as well as intranuclear filaments. In contrast, the mutant lamin A proteins responsible for PLD were found to be concentrated in disk-like or punctate structures at the nuclear periphery. No diffuse lamina staining or intranuclear filaments were observed.

This is the first demonstration of altered lamin A function resulting from disease causing mutations. Furthermore, these results support a model where expression of dominant negative lamin proteins is responsible for the observed disease phenotypes.

**Identification of two novel missense mutations in the *CSX/NKX2-5* gene encoding a cardiac specific transcription**

**factor.** *I. Gutierrez-Roelens*<sup>1</sup>, *Th. Sluysmans*<sup>2</sup>, *K. Devriendt*<sup>3</sup>, *M. Gewillig*<sup>4</sup>, *M. Vikkula*<sup>1</sup>. 1) Lab. of Human Molec. Genetics, Christian de Duve Institute, Brussels, Belgium; 2) Division of Pediatric Cardiology, Universite catholique de Louvain, Brussels, Belgium; 3) Center for Human Genetics, KUL, Leuven, Belgium; 4) Division of cardiology, KUL, Leuven, Belgium.

Non-syndromic cardiac septation defects are common, yet the responsible genetic changes remain uncharacterised. However mutations in the *CSX/NKX2-5* gene encoding a cardiac specific homeobox transcription factor have been reported to cause atrial septal defects (ASD) associated with atrioventricular (AV) conduction block. The *CSX/NKX2-5* gene is expressed in cardiac muscle during embryonic, fetal and adult life, and its *Drosophila* ortholog *tinman*, is essential for the formation of the dorsal vessel. Targeted homozygous disruption of *Nkx2-5* in mice causes lethality due to failure of heart looping. In man, ten mutations in the *CSX/NKX2-5* gene have been described in individuals with variable cardiac phenotypes. The majority has ASD and/or atrioventricular conduction defects. In addition, ventricular septal defects (VSD), tetralogy of Fallot (TOF), subvalvular aortic stenosis, ventricular hypertrophy, pulmonary atresia, mitral valve abnormalities and Ebsteins anomaly are observed. Along our studies on non-syndromic familial cardiopathies, we have collected two families with some members presenting ASD and AV-block. In both families we identified a novel *CSX/NKX2-5* mutation in the homeodomain. Variable expressivity in the phenotype was observed in one of the families. Importantly, mutation carriers do not necessarily present AV-block at young age. We have also screened the *CSX/NKX2-5* gene in sporadic and familial cases of diverse cardiopathies, to estimate mutation frequency. As mutations were not found in other than the 2 families with AV-block, *CSX/NKX2-5* seems to be a rare cause of cardiopathies without conduction defect. (vikkula@bchm.ucl.ac.be).

**Somatic second hit-hypothesis is true for glomuvenous malformations.** P. Brouillard<sup>1</sup>, L.M. Boon<sup>1,2</sup>, O. Enjolras<sup>3</sup>, M. Ghassibe<sup>1</sup>, J.B. Mulliken<sup>4</sup>, M. Vikkula<sup>1</sup>. 1) Lab. of Human Molec. Genetics, Christian de Duve Institute, Brussels, Belgium; 2) Center for Vascular Anomalies, Division of Plastic Surgery, Universite catholique de Louvain, Brussels, Belgium; 3) Consultation des Angiomes, Hopital Lariboisiere, Paris, France; 4) Vascular Anomalies Center, Childrens Hospital, Harvard Medical School, Boston, MA.

Glomuvenous malformations (GVMs), *localized* defects of vascular morphogenesis, are single or multiple bluish-purple lesions that occur mainly in skin. Histologically, the distended veins present smooth muscle-like "glomus cells" in the media. GVMs are usually dominantly inherited and, with the more common mucocutaneous venous malformations, they are among the most frequent lesions in centers that specialize in treatment of vascular anomalies (Vikkula et al., 1998). In other abstracts submitted to this meeting, we describe the criteria for clinical differential diagnosis between common VMs and GVMs (Boon et al.), as well as the identification of the causative gene, glomulin, for GVMs (Vikkula et al.). As 13 of the 14 identified mutations cause premature stop codons, GVMs are likely to be caused by loss-of-function of glomulin. This guided us to test the hypothesis that these *localized* inherited vascular lesions are due to local complete lack of glomulin function. This might be due to a somatic second hit, as was suggested for retinoblastoma by Knudson's double-hit hypothesis. Thus, we screened for somatic mutations in GVM lesions resected from families with a characterized inherited mutation. Here we report on the identification of a mutation that was different from the inherited genetic alteration, and that was not seen in genomic DNA extracted from blood. Thus, it is a *de novo* somatic mutation in DNA of a GVM lesion. These data demonstrate that GVMs are due to complete *localized* loss of glomulin function. (vikkula@bchm.ucl.ac.be).



**STRUCTURAL AND FUNCTIONAL DEFECTS OF DESMIN CAUSING CARDIAC AND SKELETAL MYOPATHY.** *A. Dagvadorj<sup>1</sup>, K-Y. Park<sup>1</sup>, V.J. Ferrans<sup>1</sup>, N. Sambuughin<sup>2</sup>, K. Sivakumar<sup>2</sup>, M.C. Dalakas<sup>1</sup>, L.G. Goldfarb<sup>1</sup>.* 1) NINDS, National Institutes of Health, Bethesda, MD; 2) Barrow Neurological Institute, Phoenix, AZ.

Desmin myopathy is characterized by amorphous accumulation of desmin and other myofibrillar proteins in the sarcoplasm of cardiac and skeletal muscle fibers. Desmin is a cytoskeletal protein in interconnecting myofibrils and attaching them to other cellular structures. We identified nine point mutations at the C-terminal part of the alpha-helical rod that is critically important for filament assembly. Six of the nine mutations introduce proline that is known to disrupt the intermediate filament network. In addition, two families had splice site mutations; in both, desmin gene was lacking exon 3 leading to an in-frame deletion of 32 amino acids and making desmin molecule non-functional. Most of the studied families (9 of 13) show autosomal dominant pattern of inheritance. Patients in one family had compound heterozygosity with amino acid-altering point mutations on both desmin alleles. Three our patients were sporadic; neither of their unaffected parents showed desmin mutations, thus confirming that the mutations have occurred de novo. To demonstrate the functional consequences of desmin mutations we expressed wild type and mutant cDNA in SW13 cells and studied them by immunocytochemistry. SW13 cells are of epithelial origin and normally do not express desmin or other intermediate filaments. Cells transfected with wild type cDNA produced functional desmin that was capable of forming an intermediate filament network. Mutant desmin was unable to build an intermediate filament network and instead aggregated into clumps of desmin-reactive material scattered throughout the cytoplasm. The presence of desmin mutations in patients with desmin-related cardiac and skeletal myopathy clustered at the highly conserved C-terminal end of the desmin rod domain that plays an important role in filament assembly, and functional analysis of these mutations demonstrating the inability of mutant desmin molecules to construct a filament network, strongly suggest that the mutations we have identified in the desmin gene are implicated in the phenotypes observed in the affected families.

**Automated mutation screening using dideoxy fingerprinting and capillary array electrophoresis.** *P.S. Andersen<sup>1</sup>, M. Johnson<sup>2</sup>, C. Brown<sup>2</sup>, M. Christiansen<sup>1</sup>, R. Frank-Hansen<sup>1</sup>, J. Vuust<sup>1</sup>, L.A. Larsen<sup>3</sup>.* 1) Dept. of Clinical Biochemistry, Statens Serum Institut, Copenhagen, Denmark; 2) Applied Biosystems, Foster City, CA; 3) Dept. of Medical Genetics, University of Copenhagen, Copenhagen, Denmark.

The rapid progress in the isolation of genes associated with human disease has resulted in an increasing demand for mutation screening methods. The molecular diagnosis of the long QT syndrome (LQTS) - a cardiac disorder characterized by prolongation of the QT<sub>c</sub> interval in the ECG, syncope and sudden death - requires mutation screening of all exons in at least five genes, encoding cardiac Na<sup>+</sup> and K<sup>+</sup> channel subunits.

A method for automated dideoxy fingerprinting (ddF) using capillary array electrophoresis (CAE) was developed and the efficiency of the method was tested by analyzing 24 DNA samples with mutations in one of the genes *KCNQ1* and *KCNH2*, which are involved in 50% of LQTS cases. One of these mutations, 362insQK in *KCNQ1*, is novel.

The sensitivity was 100% using a single electrophoresis temperature of 18 °C or 25 °C. However, analysis of the samples in both the sense and anti-sense direction were required for high sensitivity. Analysis in a single direction resulted in a decrease of the sensitivity to 74% and 70%, respectively.

The throughput of the ddF method, if performed with a 16 capillary CAE instrument is 288 samples per 7 hours if each sample is analyzed on both strands.

**Familial thoracic aortic aneurysms and dissections : genetic heterogeneity with a major locus mapping to 5q13-**

**14.** *D. Guo<sup>1</sup>, R. He<sup>1</sup>, S.N. Hasham<sup>1</sup>, S. Shete<sup>2</sup>, D.M. Milewicz<sup>1</sup>*. 1) Dept Internal Medicine, Univ Texas/Houston Med Sch, Houston, TX; 2) Dept of Epidemiology, M.D.Anderson Cancer Centre, Houston, TX.

Thoracic aortic aneurysms and dissections (TAAs) are the major disease process affecting the aorta. Previous study on 15 TAAs families mapped a major locus to 7.8cM critical interval on 5q13-14 with a maximum LOD score ( $Z_{max}$ ) of 4.74. An STS tagged sequence based BAC contig was assembled for the critical region on 5q. Dinucleotide repeats were identified in the sequenced BACs of the critical interval and used for fine genotyping. Fine mapping was carried out in family TAA013 that had an affected individual with double recombination over the critical interval. The results suggest that the critical interval containing the defective gene lie in an interval of 2cM. Eleven new families with TAAs were recruited that did not show linkage to FBN1, one of which showed linkage to 5q. With this, the maximum LOD score for marker D5S2029 has increased to 5.6. All of these families were genotyped with the microsatellite markers surrounding the candidate loci at 3p and 11q. By haplotype analysis, five of families did not show evidence of linkage to markers on 3p, 5q or 11q. This indicates that there is at least one more locus for familial TAAs disease.

**The genetics of long QT syndrome: Mutation detection in 100 families.** *M. Christiansen*<sup>1</sup>, *L.A. Larsen*<sup>2</sup>, *R.F. Hansen*<sup>1</sup>, *P.S. Andersen*<sup>1</sup>, *J. Vuust*<sup>1</sup>, *J.K. Kanters*<sup>3</sup>, *L. Tranebjaerg*<sup>4</sup>, *G. Wettrell*<sup>5</sup>. 1) Dept Clinical Biochemistry, Statens Serum Inst, Copenhagen, Denmark; 2) Institute of Medical Biochemistry and Genetics, University of Copenhagen, Denmark; 3) Dept Medical Physiology, University of Copenhagen, Denmark; 4) Dept of Medical Genetics, Tromsø University Hospital, Tromsø; Norway; 5) Dept of Pediatrics, University Hospital, Lund.

100 families with long QT syndrome (LQTS), a hereditary cardiac disease characterized by prolonged cardiac repolarization and syncopes, polymorphous ventricular tachycardia and sudden death, were examined for mutations in genes coding for cardiac ion channels using SSCP-HD. In *KCNQ1* 19 mutations were found in 23 families, and in *KCNH2* 23 mutations were found in 24 families, whereas no mutations were found in neither *KCNE1* nor *KCNE2*. Three novel mutations in *SCN5A*, IVS5DS, V411M, and V1251M, were found in three families. Nearly all mutations were private and 75% were missense mutations. As prolonged repolarisation can theoretically be caused by changes in the transient outward potassium current (I<sub>to</sub>) conducted by ion channels coded for by the genes *KCDN2* and *KCDN3*, we examined whether mutations in the latter gene could be found in 23 families with no other mutations identified. As no mutations were found we conclude that mutations affecting I<sub>to</sub>, as well as mutations affecting I<sub>Na</sub>, conducted by *SCN5A*, are at most a rare cause of LQTS. A large number of common and rare polymorphisms were identified in the LQTS associated genes and their significance in propensity for arrhythmias and drug-induced proarrhythmia should be examined in large epidemiological studies. Furthermore, we still need to identify the genetic substrate for the disease in the 50% of familial LQTS cases where we do not find mutations in the known LQTS-associated genes.

**Sarcoplasmic calcium channel mutations in inherited and acquired forms of human ventricular arrhythmias.** *P.J. Laitinen*<sup>1,2</sup>, *H. Fodstad*<sup>1,2</sup>, *H. Swan*<sup>1</sup>, *K. Paavonen*<sup>1,2</sup>, *K. Piippo*<sup>1,2</sup>, *M. Viitasalo*<sup>1</sup>, *L. Toivonen*<sup>1</sup>, *N. Rosembli*<sup>3</sup>, *A.R. Marks*<sup>3</sup>, *K. Kontula*<sup>1,2</sup>. 1) Department of Medicine, University of Helsinki, Helsinki, Finland; 2) Program of Molecular Medicine, Biomedicum Helsinki, Helsinki, Finland; 3) Center for Molecular Cardiology, Columbia University, New York, USA.

Familial polymorphic ventricular tachycardia (FPVT) is a highly malignant, autosomally dominantly inherited disorder. It manifests with bidirectional and polymorphic ventricular tachycardias in response to vigorous exercise. No structural abnormalities of the heart are observed. We recently identified that the gene underlying FPVT is the cardiac ryanodine receptor gene (RYR2) which encodes a sarcoplasmic calcium channel. Point mutations in the RYR2 gene were found in three of the four known Finnish FPVT families. In addition two amino acid polymorphisms were detected. As an extension to these initial studies, we aim at characterizing the specific effects of the recently identified mutations and polymorphisms. Our second aim is to find out whether these alterations could predispose to arrhythmic disorders other than FPVT. To this end, specific restriction fragment length polymorphism assays for each DNA alteration were set up. Functional studies will be conducted using in vitro mutagenesis and patch clamp technique. We have expanded our FPVT pedigrees, and DNA samples of several new individuals from each FPVT family were added to the study. At present, the number of affected and non-affected individuals in Finnish FPVT families are as follows: P2328S 22 and 35, Q4201R 3 and 22, V4653F 11 and 26, respectively. We are currently screening RYR2 polymorphisms and mutations in several well documented clinical cohorts consisting of patients with ventricular fibrillation due to ischemic heart disease and other cardiac disorders as well as individuals undergoing sudden death. The prevalence of the HERG ion channel polymorphism K897T, associated with phenotypic effects on the cardiac repolarization duration is also being determined in these patients. We hypothesize that common RYR2 and HERG polymorphisms may constitute genetic markers of liability to ventricular fibrillation and cardiac sudden death.

**Comprehensive mutational analysis of the Lamin A/C gene in familial and sporadic dilated cardiomyopathies.** *L. Mestroni*<sup>1</sup>, *M.R.G. Taylor*<sup>1</sup>, *M. Robinson*<sup>1</sup>, *G.L. Brodsky*<sup>1</sup>, *A. Moss*<sup>1</sup>, *J.L. Goodnight*<sup>1</sup>, *G. Sinagra*<sup>2</sup>, *A. Di Lenarda*<sup>2</sup>, *P. Fain*<sup>1</sup>. 1) Cardiovascular Institute, University of CO, Aurora, CO; 2) University Hospital, Trieste, Italy.

**Background:** Mutations in the lamin A/C gene (*LMNA*) have been described in patients with dilated cardiomyopathy (DCM). The overall spectrum, the frequency, and the genotype/phenotype correlation of *LMNA* mutations in this condition remain unknown. **Methods:** Fifty-two families of DCM index patients from different ethnic groups were screened for *LMNA* mutations: 43 familial DCM and 9 non-familial (sporadic). DNA from 76 affected family members was screened for mutations in *LMNA*. Denaturing high performance liquid chromatography (DHPLC) was utilized for mutation analysis. **Results:** Putative *LMNA* mutations were detected in four nuclear families: exon 1 (Arg89Leu), exon 6 (delT959 and Arg377His) and exon 11 (Ser573Leu), respectively. The delT959 mutation is predicted to lead to a frameshift and novel carboxy-terminal protein sequence. The exon 11 missense mutation was present in a sporadic case. The Arg89Leu and the Ser573Leu mutations produced a DCM phenotype, with complicating arrhythmia, but no muscle or conduction defects. In both families with exon 6 mutations, there was significant inter- and intra-familial phenotypic variability, ranging from complete absence to overt signs of muscular dystrophy (Emery-Dreifuss or limb-girdle muscular dystrophy). Four patients had elevated creatine kinases and the 2 probands had abnormal muscle biopsies. Cardiac involvement included conduction abnormalities (6 cases) and atrial and/or ventricular arrhythmia in all patients. The mutated residues are highly conserved across differing species, the mutations were not observed in more than 150 chromosomes, and, in the familial cases, cosegregated with the disease. In addition, a number of intronic and 4 exonic single nucleotide polymorphisms were identified. **Conclusions:** These results suggest that *LMNA* mutations can cause DCM in a relevant proportion of familial and non-familial cases. The results also illustrate the variability in skeletal muscle disease phenotype that characterizes *LMNA* mutations.

**Genetic risk for sudden cardiac death: ethical issues of molecular testing for hypertrophic cardiomyopathy.** V. Meiner<sup>1</sup>, M.Y. Flugelman<sup>2</sup>, A. Lorber<sup>3</sup>, D. Stern<sup>4</sup>, S. Shpitzen<sup>4</sup>, L. Ben-Avi<sup>4</sup>, A. Keren<sup>5</sup>, E. Leitersdorf<sup>4</sup>. 1) Dept of Human Genetics, Hadassah Univ Hosp, Jerusalem, Israel; 2) Dept of Cardiology, Lady Davis Carmel Medical Center, Haifa, Israel; 3) Dept of Pediatric Cardiology, Rambam Medical Center, Haifa, Israel; 4) Center for Research, Prevention, and Treatment of Atherosclerosis, Hadassah University Hospital, Jerusalem Israel; 5) The Heiden Department of Cardiology, Bikur Cholim Hospital, Jerusalem, Israel.

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disorder associated with a variable clinical course. Possible treatments include change in life style, implantation of a cardioverter defibrillator, and drug treatment. A considerable inter- and intra-familial diversity is emphasized by the fact that HCM may be clinically evident in all stages of life. Moreover, some patients remain asymptomatic throughout their lives, others develop severe symptoms of heart failure, and some die suddenly in the absence of antecedent symptoms. The impact of molecular genetics in the diagnosis of HCM is continuously increasing, leading to improved understanding of various aspects of its pathophysiology. Yet, important questions are raised concerning the practical significance of DNA testing in HCM. In order to assess the potential merit of molecular genetics in HCM, we studied nine Israeli families and reviewed our experience. DNA samples from individuals with either familial or sporadic HCM were analyzed and specific mutations in sarcomeric genes were characterized. The value of genetic counseling as an integral part of the treatment in HCM is emphasized. In one of the families, prenatal diagnosis through CVS was applied and resulted in termination of the pregnancy. The dilemma of whether to ground therapeutic considerations on molecular findings in an uncertain phenotype within family members is constantly raised. Unpredicted disease course with possible sudden death as a presenting symptom combined with desperate families seeking for an active treatment, in contrast to the uncertain significance of the mutation without clinical findings, raises ethical questions as to the current role of molecular genetics in HCM.

**Detection of Sequence Variations in the LDL-Receptor Gene Using Denaturing High Performance Liquid Chromatography.** *S. Morrow<sup>1</sup>, M. Robinson<sup>1</sup>, M. Volkova<sup>2</sup>, E.I. Schwartz<sup>2</sup>, M.A. Marino<sup>2</sup>.* 1) Applied Genomics & Molecular Genetics, Transgenomic, Inc., Omaha, NE; 2) Applied Genomics & Molecular Genetics, Transgenomic, Inc., Gaithersburg, MD.

The low-density lipoprotein (LDL)-receptor gene contains a high degree of allelic heterogeneity. A variety of mutations at this locus result in the development of familial hypercholesterolemia (FH), an autosomal dominant lipoprotein metabolism disorder characterized by high cholesterol levels in the blood and premature coronary arteriosclerosis. This study utilized denaturing high performance liquid chromatography (dHPLC) to detect sequence variations in a panel of 28 patient samples with a clinical diagnosis of FH. PCR amplicons were designed for LDL-receptor exons 1-3 and 5-18, as well as the 5-UTR. DHPLC analysis demonstrated that all 28 patients were positive for a putative sequence variant in at least one exon. The DNA sequence variants are presently being confirmed by direct sequencing of PCR products. The use of dHPLC analysis utilizing the WAVE® system proved to be a useful tool for detecting both well-characterized as well as unknown sequence variations in the LDL-receptor gene.



**The WAVE® nucleic acid fragment analysis system: A versatile analytical tool for mutation detection. M.**

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The WAVE® Nucleic Acid Fragment Analysis System is a fully automated DNA analysis system that integrates denaturing high performance liquid chromatography (dHPLC) with the WAVEMaker® DNA melting prediction software and the DNASep® column. The WAVE® is an analytical tool, which excels at screening for unknown sequence variations. A few of the many disorders for which the WAVE® dHPLC system has been instrumental in the identification of previously unknown sequence variants include Holt-Oram syndrome which is caused by mutations in the T-box transcription factor gene TBX5, Familial long QT syndrome, or LQTS, a polygenic disorder involving the KVLQT1, HERG, and SCN5A genes, as well as the gene involved in the development of Neurofibromatosis type 2 (NF2). The automated high-throughput capability, high sensitivity of detection compared to other methods, and ease of data analysis have made the WAVE® the method of choice for high throughput SNP screening and mutation detection projects.

**CFC1 as a candidate gene for congenital cardiovascular malformations.** *J.D. Karkera<sup>1</sup>, A.K. Kantipong<sup>2</sup>, E. Roessler<sup>1</sup>, R.N. Bamford<sup>1</sup>, J. dela Cruz<sup>1</sup>, J.A. Towbin<sup>3</sup>, P. Bowers<sup>4</sup>, R. Burdine<sup>5</sup>, A. Schier<sup>5</sup>, E. Goldmuntz<sup>6</sup>, M. Muenke<sup>1</sup>.* 1) Medical Genetics Br, NHGRI/NIH, Bethesda, MD; 2) HHMI-NIH Scholar, MGB, NHGR/NIH, Bethesda, MD; 3) Baylor College of Medicine, Houston, TX; 4) Yale University, New Haven, CT; 5) Skirball Institute of Biomolecular Medicine, NY; 6) The Children's Hospital of Philadelphia, PA.

In vertebrates, the organs of chest and abdomen have a specific non-random asymmetric arrangement with respect to the midline of the body called Left-Right (L-R) asymmetry. Several reports have suggested that some cardiac abnormalities might be attributable to mutations in developmental control genes responsible for L-R asymmetry. Recently, mutations in CFC1 (encoding the CRYPTIC protein) have been implicated as a cause of laterality defects and transposition of the great arteries (TGA) in humans. CFC1 is a member of the EGF-CFC gene family. Studies of murine CFC1 and the zebrafish homologue *oep* have suggested that these factors play a critical role in the establishment of L-R axis. In this study we have decided to screen CFC1 for mutations using PCR-dHPLC in our panel of 310 patients with a wide spectrum of cardiac anomalies: tetralogy of fallot, truncus arteriosus, interrupted aortic arch, and AV canal. We have identified several SNPs, and missense mutations. Functional complementation of zebrafish embryos defective in *oep* function are in progress to assess the effect of these variants.

**Novel mutations in three families with dilated cardiomyopathy and conduction system disease cluster in the rod domain of lamin A/C.** *P.M. Jakobs<sup>1</sup>, H.L. Keegan<sup>1</sup>, E.L. Hanson<sup>1</sup>, M. Litt<sup>2</sup>, R.E. Hershberger<sup>1</sup>.* 1) Department of Medicine, Oregon Health & Sciences University, Portland, OR 97201, USA; 2) Department of Molecular and Medical Genetics, Oregon Health & Sciences University, Portland, OR 97201, USA.

Mutations in inherited forms of dilated cardiomyopathy account for at least 35% of cases of idiopathic dilated cardiomyopathy. Several autosomal dominant inherited, familial dilated cardiomyopathy (FDC) disease genes  $\alpha$ -cardiac actin, desmin, d-sarcoglycan,  $\beta$ -myosin heavy chain, troponin T,  $\alpha$ -Tropomyosin and lamin A/C - have been identified. Lamin A/C are alternatively spliced nuclear envelope proteins encoded by the LMNA gene. Mutations in LMNA are responsible for various pathologies: Emery-Dreifuss muscular dystrophy (EDMD2), limb girdle muscular dystrophy type 1B (LGMD1B), Dunnigan type familial partial lipodystrophy (FPLD) and dilated cardiomyopathy with conduction defects (CMD1A). Detailed phenotypic reports and the identification of novel mutations in distinct areas of this intermediate filament protein could give additional insight into the function of lamin A/C. We identified novel LMNA mutations in the rod domain of lamin A/C by direct sequencing of LMNA exons, including the exon / intron junctions, in one affected individual from sixteen large families. Variants were confirmed by either allele specific oligohybridizations (ASOs) or restriction digests (RFLPs). A missense mutation (E203K) was identified in 14 affected individuals of family A. Disease presented as progressive conduction disease in the 4th and 5th decades of life. A nonsense mutation (R225X) in ten adult subjects of family B showed progressive conduction disease with an average of one decade earlier onset of ventricular arrhythmias, left ventricular enlargement and systolic dysfunction. In family C, a missense mutation (L215P) was identified in seven adult subjects with disease onset in the 4th and 5th decades. This data, together with previous reports, suggest that lamin A/C mutations may account for 10-20% of cases of FDC.

**Mutations in cardiac actin appear to be rare in dilated cardiomyopathies.** R. Zolty<sup>1</sup>, G. Passarino<sup>2</sup>, G.L. Brodsky<sup>1</sup>, M.R.G. Taylor<sup>3</sup>, A. Moss<sup>1</sup>, M.R. Bristow<sup>1</sup>, L. Cavalli-Sforza<sup>2</sup>, P.A. Underhill<sup>2</sup>, L. Mestroni<sup>1</sup>. 1) CU-Cardiovascular Institute, UCHSC at Fitzimons, Aurora, CO; 2) Department of Genetics, Stanford University, Palo Alto, CA; 3) University of Colorado Health Sciences Center, Denver, CO.

**Background:** Idiopathic dilated cardiomyopathy (DCM) is frequently inherited and genetically heterogeneous. Previous investigators have reported mutations in exons 5 (Arg312His) and 6 (Glu361Gly) of the cardiac actin gene (*ACTC*) in two families with DCM. **Methods:** We designed a study to evaluate the prevalence and characteristics of *ACTC* gene mutations in DCM. 65 DCM patients from different ethnic backgrounds were studied: 48 had familial DCM (from 32 unrelated families) and 17 had sporadic DCM. The controls were two patients with ischemic heart disease. Genomic DNA was extracted from blood or explanted heart tissue using standard procedures. PCR products were produced from all 6 exons of the *ACTC* gene, allowing the inclusion of the exon/intron boundaries. The G-to-A transversion in codon 312 (Arg312His) of exon 5 introduces a unique Bcl1/Nde1 restriction site. Mutation analysis of all 6 exons was performed using denaturing high performance liquid chromatography (DHPLC) and sequence analysis. In addition, Exon 5 amplicons were digested with Bcl1/Nde1. **Results:** The mutant restriction fragments were not detected in any of the exon 5 amplicons. The DHPLC evaluation uncovered a single nucleotide polymorphism, which was confirmed by sequence analysis to be in intron 5 (C-62T). No genetic variations could be found in the other exons. **Conclusions:** *ACTC* mutations were not associated with DCM in our large population of familial and sporadic DCM. *ACTC* mutations appear to be only rarely associated with DCM.

**Haploinsufficiency for one *COL3A1* allele of type III procollagen results in a phenotype similar to the vascular form of Ehlers-Danlos syndrome (EDS type IV).** *U. Schwarze*<sup>1</sup>, *W.I. Schievink*<sup>2</sup>, *E. Petty*<sup>3</sup>, *M.R. Jaff*<sup>4</sup>, *D. Babovic-Vuksanovic*<sup>5</sup>, *K.J. Cherry*<sup>5</sup>, *M. Pepin*<sup>1</sup>, *P.H. Byers*<sup>1</sup>. 1) University of Washington, Seattle, WA; 2) Cedars-Sinai Medical Center, Los Angeles, CA; 3) University of Michigan, Ann Arbor, MI; 4) The Heart and Vascular Institute, Morristown, NJ; 5) Mayo Clinic, Rochester, MN.

Mutations in the *COL3A1* gene that encodes the chains of type III procollagen result in the vascular form of the Ehlers-Danlos syndrome, if they alter the sequence in the triple helical domain. Although mutations in other fibrillar collagen genes that lead to allele instability or failure to incorporate pro- $\alpha$  chains into trimer and thus reduce the amount of the mature molecule produced result in clinically apparent phenotypes, no such mutations in the *COL3A1* gene have been identified. Further, mice heterozygous for *Col3a1* "null" alleles have no identified phenotype.

We have now found three frame-shift mutations (1832delAA, 413delC, and 555delT) that led to premature termination codons (PTC) in exons 27, 6 and 9, respectively, and to allele product instability. The mRNA from the mutant allele was transcribed efficiently but rapidly degraded, presumably by the mechanisms of nonsense-mediated decay. In a fourth patient we identified a point mutation in the final exon (exon 52) that resulted in a PTC (4294C $\rightarrow$ T; Arg1432Ter). In this last instance the mRNA was stable but led to synthesis of a truncated protein that was not incorporated into the mature type III procollagen molecule. In all probands the presenting feature was vascular aneurysm or rupture. Thus, in contrast to mutations in genes that encode the dominant protein of a tissue (e.g., *COL1A1* and *COL2A1*) in which "null" mutations result in milder phenotypes than those that alter protein sequence, the phenotypes produced by these mutations in the *COL3A1* gene overlap with those of the vascular form of EDS. This suggests that the major effect of many of these dominant mutations in the "minor" collagen genes may be expressed through protein deficiency rather than incorporation of structurally altered molecules into fibrils.

**A novel mutation in the *SDHD* gene in a family with inherited paragangliomas.** *L. Renard*<sup>1</sup>, *C. Godfraind*<sup>2</sup>, *L.M. Boon*<sup>3,4</sup>, *M. Vikkula*<sup>4</sup>. 1) Dept. of Radiotherapy, Cliniques univ. St Luc, Univ. catholique de Louvain, Brussels, Belgium; 2) Lab. of Neuropathology, Cliniques univ. St Luc, Univ. catholique de Louvain, Brussels, Belgium; 3) Div. of Plastic Surgery, Centre for Vascular Anomalies, Cliniques univ. St Luc, Univ. catholique de Louvain, Brussels, Belgium; 4) Lab. of Human Molecular Genetics, Christian de Duve Institute & Univ. catholique de Louvain, Brussels, Belgium.

Paragangliomas (OMIM# 168000), (carotid body tumours) are vascularized tumors of the head and neck. These tumors may be unilateral, although in families with inherited predisposition, they tend to be bilateral and multiple. It has been observed that paragangliomas are inherited almost exclusively via the paternal line; thus maternal imprinting occurs. This helped in finding linkage to *11q23-qter* (Heutink et al. 1992). Positional candidate gene analysis recently resulted in the identification of the mutated gene, *SDHD* that encodes the small subunit of cytochrome b in the succinate-ubiquinone oxidoreductase complex (*PGL1*) (Baysal et al. 2000). This enzyme complex is important for the tricarboxylic acid cycle and the aerobic respiratory chains in mitochondria. Thus, *SDHD* is likely to be critical for the oxygen-sensing system, and its loss may lead to chronic hypoxic stimulation of cellular proliferation that leads to tumorous growth. Interestingly, another *PGL* locus has been mapped more telomerically on 11q (*PGL2*), and a mutation in the *SDHC* gene (*PGL3*) was identified in a family with non-maternally imprinted inheritance of paragangliomas (Niemann, and Muller, 2000). We studied a family with three generations affected with paragangliomas. Generation skipping of the phenotype was noted once, when the disorder seemed to be inherited from the mother. Thus, the *SDHD* gene became the most likely candidate. In fact, we identified a novel splice site mutation that co-segregated with the phenotype. Interestingly, six non-affected carriers were observed, and all of them had inherited the mutant allele from their mother. The identification of carriers enables genetic counseling, an important aspect for these usually treatable vascular tumors. (vikkula@bchm.ucl.ac.be).

**A new phenotype of familial visceral inversus/ atrial fibrillation/ ASD caused by a novel 7bp deletion in the CSX/NKX2.5 gene.** *Y. Watanabe*<sup>1,2</sup>, *S. Yano*<sup>2,3</sup>, *T. Akagi*<sup>2</sup>, *M. Yoshino*<sup>2</sup>, *J.C. Murray*<sup>1</sup>. 1) Department of Pediatrics, University of Iowa, Iowa City, IA; 2) Department of Pediatrics and Child Health, Kurume University School of Medicine, Fukuoka, Japan; 3) Medical Genetics, Department of Pediatrics, Childrens Hospital Los Angeles, University of Southern California, Los Angeles, CA.

*CSX/NKX2.5* (Cardiac Specific Homeobox) is homologous to mouse gene *NKX2.5* and plays an important role in early cardiac development. Mutations in the *CSX/NKX2.5* have been reported in individuals with AV node conduction failure and/or different cardiac structural anomalies, especially atrial septal defect (ASD). We identified a novel 7-bp deletion in exon 1 at nucleotide +215 from the translation starting point of the *CSX/NKX2.5* gene in a familial case of ASD, resulting in a frameshift of downstream codons. The mutation cosegregated with ASD and/or atrial-ventricular conduction abnormality as an autosomal dominant in four members in two generations. The proband had visceral inversus, polysplenia with symmetrical liver and an ASD. The younger sister of the proband was a carrier of this mutation and was diagnosed with atrial fibrillation (Af) as the only phenotypic expression of the gene mutation. This is the first report in humans that may suggest that the *CSX* gene is involved in the regulation of Left-Right axis determination and that *CSX* gene mutations can cause Af. In a previous phenotype-genotype correlation study (Kawahara, H., et al., *Journal of Clinical Investigation*, 2000. 106(2): p. 299-308) it was suggested that loss of function caused AV conduction delay but that hypomorphic mutant proteins cause cardiac anatomical anomalies such as ASD, VSD and TOF. The family reported here has a mix of conduction and structural defect, suggesting a role for genetic, stochastic or environmental modifiers. Searches for additional mutations in similarly affected individuals will be important.

**Iron metabolism in genetically iron overloaded frataxin deficient mice.** *M. Santos, C. Miranda, M. Pandolfo.* Centre de Recherche, Centre Hospitalier de l'Universite de Montreal, Montreal, Quebec, Canada.

Friedreich's ataxia (FA) is the most common form of autosomal recessive spinocerebellar ataxia. The disease is caused by an expanded intronic GAA repeat, which results in deficiency of a mitochondrial protein called frataxin. Inactivation of the frataxin mouse gene leads to early embryonic lethality (1). In humans, there is always residual frataxin expression (10-30%) and total frataxin deficiency has never been observed. In mice heterozygous for frataxin deficiency ( $Fx^{+/-}$ ), frataxin levels are 50% diminished. Some evidence indicate that frataxin deficiency leads to mitochondrial iron accumulation and associated production of oxygen free radicals. To gain insight into the pathogenesis of FA and to explore the possibility that iron overload could precipitate FA in  $Fx^{+/-}$  mice, we have bred  $Fx^{+/-}$  mice with  $Hfe^{-/-}$  knockout mice (2), an animal model of Hereditary Hemochromatosis (HH). HH is a prevalent human disease caused by a mutation in HFE, which encodes a non-classical Major Histocompatibility Complex (MHC) class I protein involved in regulation of intestinal iron absorption.  $Hfe$  deficiency causes systemic iron overload. The generated  $Hfe^{-/-}Fx^{+/-}$  mutant mice have increased serum iron and transferrin saturation. Blood erythroid parameters are normal. Iron concentration, determined by atomic absorption spectrometry, was significantly increased in the livers, a feature confirmed histologically. Motor coordination, evaluated using the rotarod test, is indistinguishable from wild type and single-mutant mice. These study shows that reduction of frataxin levels to 50% does not cause FA even in the presence of significant systemic iron overload. Gene therapy attempts to increase frataxin levels up to 50% in FA patients may thus constitute a promising approach. (1) Cossee M, Puccio H, Gansmuller A, Koutnikova H, Dierich A, LeMeur M, Fischbeck K, Dolle P, Koenig M. Inactivation of the Friedreich ataxia mouse gene leads to early embryonic lethality without iron accumulation. *Hum Mol Genet* (2000) 9:1219. (2) Levy JE, Montross LK, Cohen DE, Fleming MD, Andrews NC. The C282Y mutation causing hereditary hemochromatosis does not produce a null allele. *Blood* (1999) 94:9.



**Heterozygous b epithelial sodium channel mice exhibit features of pseudohypoaldosteronism type I (PHA I).** *R.A. Williamson<sup>1</sup>, B. Yang<sup>1</sup>, X.R. Cao<sup>1</sup>, M.J. Welsh<sup>2</sup>, J.B. Stokes<sup>2</sup>.* 1) Dept OB/GYN, and; 2) Internal Med, Univ. of Iowa Coll. of Med, Iowa City, IA.

Epithelial sodium channels (ENaC), composed of three homologous subunits (a, b, g), are key mediators of Na<sup>+</sup> balance and BP regulation. Loss of function mutations in any of the subunits cause PHA I, a salt wasting autosomal recessive condition. Our group inactivated the b-subunit of ENaC which produced perinatal death 8-48 hours after birth in affected homozygotes (McDonald FJ et al. Proc Natl Acad Sci USA 96:1727, 1999). The salient finding was a markedly elevated serum K<sup>+</sup>. Associated features included significantly elevated serum aldosterone levels, a decreased serum Na<sup>+</sup>, increased urine Na<sup>+</sup> and decreased urine K<sup>+</sup> in b ENaC <sup>-/-</sup> pups. We have studied b ENaC <sup>+/-</sup> mice utilizing a protocol which assured the controls and heterozygotes would be genetically equivalent by breeding heterozygous 129 to C57/B16 strain mice. For these experiments the carotid artery was cannulated for BP measurements and blood draws, the jugular vein was catheterized for drug and saline infusion, and the bladder catheterized for urine sampling. A minimum of five mice (age 2-3 months) per group was studied. No significant differences between heterozygotes and <sup>+/+</sup> controls were documented in serum aldosterone levels, serum and urine Na<sup>+</sup> and K<sup>+</sup>, resting BP, BP response to infused NaCl and KCl, and BP response to increasing doses of infused angiotensin II. However, whereas serum Na<sup>+</sup> and urine Na<sup>+</sup> were not altered in response to a normal saline volume load (5% of body weight infused over 30 minutes) or K<sup>+</sup> load (0.13 mEq/ml of KCl in normal saline in a volume equal to 5% of body weight infused over 30 minutes), we documented a significant decrease in the rate and total amount of urine K<sup>+</sup> excretion, and a decreased rate of clearance of serum K<sup>+</sup> in heterozygotes administered these challenges. Thus, b ENaC heterozygotes replicate some of the findings associated with PHA I. This study suggests that provocative testing of these heterozygous knockout mice reveals an otherwise undetectable defect in K<sup>+</sup> secretion.

**Complete glucokinase deficiency is not a common cause of permanent neonatal diabetes in European cases of neonatal diabetes.** *A.L. Gloyn<sup>1</sup>, S. Ellard<sup>1</sup>, J.P.H. Shield<sup>2</sup>, I.K. Temple<sup>3</sup>, D.J.G. Mackay<sup>3</sup>, T. Barrett<sup>4</sup>, A.T. Hattersley<sup>1</sup>.*  
1) Centre for Molecular Genetics, University of Exeter; 2) Royal Hospital for Sick Children, Bristol; 3) Wessex Regional Genetics Service and Laboratory, Salisbury; 4) Birmingham Childrens Hospital, UK.

Neonatal diabetes mellitus (NDM) is a rare disorder with an estimated incidence of 1 in 400,000. It is defined as insulin requiring hyperglycaemia within the first month of life. NDM is heterogeneous and can be either transient (TNDM) if resolved within 18 months or permanent (PNDM). Recently, 2 cases were reported where PNDM was found to result from homozygous mutations in the glucokinase gene resulting in complete absence of the enzyme glucokinase, a key regulator of glucose metabolism in pancreatic beta - cells. Heterozygous mutations in glucokinase (GCK) cause maturity onset diabetes of the young (MODY 2) which result in stable mild fasting hyperglycaemia. We studied the GCK gene in 12 cases of PNDM from a European collection to determine whether complete glucokinase deficiency is a common cause of PNDM. The coding regions and the intron-exon boundaries of the GCK gene were amplified and sequenced on an ABI 377 DNA sequencer. Sequencing identified a common previously reported polymorphism in intron 9 (IVS9 +8 c>t) but no mutations were detected. From a published series in gestational diabetes the population frequency of carriers of GCK mutations is approximately 0.0009, so we would predict a population frequency of 1/1,200,000 for compound heterozygotes or homozygotes. This may be an underestimate if there are consanguineous MODY 2 families. In conclusion homozygous or compound heterozygous glucokinase mutations are not a common cause of PNDM despite the fact that theoretically they might be predicted to account for up to 40% of cases of NDM.

**Autosomal recessive colobomatous micro/anophthalmia in a consanguineous Irish traveller family - further genetic heterogeneity.** *S. Ennis*<sup>1,2</sup>, *M. Ni Chroinin*<sup>2</sup>, *P. Tormey*<sup>3</sup>, *A.J. Green*<sup>1,2</sup>. 1) National centre for Medical Genetics, Dublin 12, Ireland; 2) Dept. of Medical Genetics, University College Dublin, Dublin, Ireland; 3) Dept of Ophthalmology, Waterford Regional Hospital, Waterford, Ireland.

We describe 9 members of a consanguineous Irish traveller family, with severe visual impairment due to anophthalmia, microphthalmia or colobomatous microphthalmia. Two members have complete bilateral anophthalmia, five had unilateral anophthalmia and contralateral colobomatous microphthalmia, and two had microphthalmia. There was no history of chemical exposure, no other associated malformations, and chromosomal analysis in two affected individuals was normal. There is pseudodominant inheritance in one family, with an affected son born to an affected father in a consanguineous marriage. Only a small number of families with autosomal recessive colobomatous micro/anophthalmia have been described before. Several reports have been of families with a consistent expression of exclusively bilateral anophthalmia in homozygotes, in contrast to the family we describe with a more varied expression in homozygotes. This is the first description of this condition in the Irish traveller population, and represents a further disease allele for this population. A previously known 14q32 locus for autosomal recessive anophthalmia, the recently described CHX10 gene on chromosome 14, and the RX gene on chromosome 18 known to cause autosomal recessive anophthalmia, have been excluded in this family. The candidate gene VSX1 has been excluded, suggesting at least a fourth locus for this condition. Homozygosity mapping for autosomal recessive anophthalmia is currently under way.

**Novel dystrophin gene mutations and unusual DMD/BMD phenotypes.** *E. Estrella*<sup>1</sup>, *R. Curless*<sup>1</sup>, *B. Roa*<sup>2</sup>, *T. Prior*<sup>3</sup>, *J. Mendell*<sup>3</sup>, *L. Baumbach*<sup>1</sup>. 1) Div Genetics, Univ. of Miami, Miami, FL; 2) Baylor College of Medicine, Houston TX; 3) Ohio State Univ., Columbus, OH.

Since the discovery of the Duchenne Muscular Dystrophy (DMD) gene, hundreds of patients with suspected primary dystrophinopathy have been evaluated for mutations. Knowledge gained from these studies has led to general genotype: phenotype predictions, although these do not account for the clinical variability noted in these disorders. Detection of novel mutations may provide new insights into functional aspects of dystrophin and/or associations with unusual clinical features. We describe patients who fall into one of these categories. The first two patients are presumed new mutations based on family history. Patient 1, 11 1/2 year-old Caucasian male, was referred for a long history of undefined weakness and unsteadiness. He had moderate elevations in serum CK. He has no pseudohypertrophy, no significant weakness, a modified Gowers maneuver, and normal reflexes. His only clinical complaint is of pain/cramps after strenuous exercise. Mutation studies revealed a DMD deletion spanning exons 3-4. This deletion has been reported in three other Becker patients. Patient 2, 6 1/2 year old African-American male, whose most significant finding is marked developmental delay for all motor and cognitive milestones since infancy, which is not found in any other family members. He is noted to have minimal muscle involvement, mild calf pseudohypertrophy, no ambulatory problems, but markedly elevated serum CKs levels. DNA studies revealed a novel exon 10-12 duplication, in conjunction with a reported African-American exon 8/9 RFLP. This patient represents a new mutation which resulted in a gross DMD gene rearrangement. His phenotype is unusual for the marked level of mental impairment. Patients three-five are classical BMD patients who share a common exon 45-53 in-frame deletion. Western blot analysis of muscle dystrophin revealed a normal level of the truncated, mutant form. DMD/BMD deletions which produce quantitatively normal dystrophin levels should be further investigated. It is our hope that similar patient descriptions will lead to further understanding of these disorders.

***CBFA1* mutations and polymorphisms in two Mexican patients with cleidocranial dysplasia.** *N. Monroy, L.E. Machuca-Tzili, S. Kofman.* Genética, Hospital General México/Facultad Medicina UNAM. México, D.F.

Cleidocranial dysplasia (CCD) is an autosomal dominant skeletal disorder whose phenotypic characteristics embrace a wide spectrum from classical cases with endochondral ossification problems to mild ones presenting neonatal or supernumerary teeth as the unique manifestation. It has been demonstrated that haploinsufficiency of *CBFA1* (located on chromosome 6p21) causes CCD, and several patients with deletions, insertions, missense and nonsense mutations of this gene have been described. In order to identify mutations in *CBFA1*, we analyzed genomic DNA from two unrelated patients with the typical craniofacial features of CCD syndrome. The DNA fragments from exon 0 to exon 7 of *CBFA1* were amplified by PCR and directly automated sequenced. Our results showed two novel mutations and three polymorphisms not previously described. One case showed a (Gln-Leu) change in exon 1, inside the Q/A domain. The other patient presented a change in exon 7 at the stop codon. In theory, the latter produces the addition of 23 aminoacids before the following termination triplet. Our findings expand the list of *CBFA1* allelic variants in CCD. We also discuss the genotype-phenotype correlations in these individuals.

**Adipocyte specific transcripts that interact with Lamin A: Implications for partial lipodystrophy.** *D.J. Lloyd, S. Shackleton, R.C. Trembath.* Division of Medical Genetics, Department of Genetics, University of Leicester, Leicester, Leicestershire, United Kingdom.

The nuclear lamina is a meshwork-like structure within the inner nuclear membrane, mainly composed of nuclear lamins. Lamin A and C are alternative transcripts of the LMNA gene at 1q21. Heterozygous mutations in LMNA have recently been reported in several inherited disorders each associated with an inability to maintain a specific cell type; these include Emery-Dreifuss muscular dystrophy, Limb-Girdle muscular dystrophy type 1B, dilated cardiomyopathy 1a or Dunnigan-type familial partial lipodystrophy (FPLD). How different mutations in LMNA can cause these different phenotypes is of much interest. Here we present the results of a yeast-two hybrid screen using mouse lamin A as bait exposed to an adipocyte cDNA library. Three categories of positive interacting clones were identified; a set of mouse lamin A clones, and a set of Sterol-regulatory element binding protein (SREBP) clones, and a set of novel transcripts. In vitro binding assays, co-immunoprecipitation of, and two-hybrid analysis of the human homologues confirmed the specificity of these interactions. SREBP exists in three known isoforms and is associated with cholesterol biosynthesis, but has also been recognised as an adipocyte differentiation factor. The novel transcript has domain homology to a nuclear membrane association protein involved in development. We therefore propose the mutations in LMNA which are associated with the FPLD phenotype interfere with these interactions, resulting in a defect in adipocyte development or maintenance.

**Establishment of a nomenclature system for X-linked amelogenesis imperfecta.** *P.S. Hart<sup>1</sup>, T.C. Hart<sup>1</sup>, J.T. Wright<sup>2</sup>*. 1) Dept Human Genetics, Univ Pittsburgh, Pittsburgh, PA; 2) Dept Pediatric Dentistry, School of Dentistry, Univ of North Carolina, Chapel Hill, NC.

The Amelogenesis Imperfecta (AI) are a group of inherited conditions that affect the development of enamel. Depending on the type of AI, the enamel defects include abnormalities in the amount, structure and composition. The molecular basis of these conditions has only been identified for the X-linked form, which is due to mutations in the X chromosome amelogenin gene (AMELX). Mutational analyses of affected individuals has begun to permit identification of genotype-phenotype correlations. For example, mutations altering the C terminus 3' of amino acid 172 result in a hypoplastic phenotype in contrast to other mutations that result in mineralization type defects. Past nomenclature systems have differed depending upon whether numbering began with the mature peptide or the signal peptide, with most beginning with the mature peptide. In addition, alternative splicing occurs, complicating the nomenclature system. As more families are analyzed, there needs to be a clearly defined nomenclature system for numbering the nucleotide and amino acid sequences. Thus, we are proposing a standard nomenclature system based on internationally agreed upon guidelines (Antonarakis et al., Hum Mutat 1998;11:1-3) using designated reference sequences. For cDNA numbering, the A of the initiator ATG is taken as +1, using NM001142 as the reference sequence. For previous numbering systems based upon the mature peptide, this will add 16 amino acids to protein designations and 48 nt to cDNA designations. As no complete genomic AMELX sequence is in the NCBI database, the authors have submitted a sequence that contains the complete genomic AMELX sequence. This sequence will serve as the reference sequence for genomic numbering. The 11 known AMELX mutations will be listed with the old and new nomenclature systems. A mutational database for AI has been established at [www.genetics.pitt.edu/AI](http://www.genetics.pitt.edu/AI).

**Identifacation and analysis of mutations in the Wilson disease gene (ATP 7B) from Indian children . R. Prasad<sup>1</sup>, G. kaur<sup>2</sup>, B.R. Thapa<sup>3</sup>.** 1) Biochemistry, PGIMER, Chandigarh-160012, U.T., India; 2) Department of Physiology, Chandigarh Medical College, Chandigarh 160034 INDIA; 3) Pediatric Gastroenterology , Postgraduate Institute of Medical Education and Research, Chandigarh 160012 INDIA.

Wilson Disease (WD) is an autosomal recessive disorder characterized by toxic accumulation of copper in the liver and subsequently in the brain and other organs. On the basis of sequence homology to known genes, the WD gene (ATP 7B) appears to be a copper transporting P-type ATPase. After performing allele specific oligonucleotide (ASO) hybridization and single strand conformational polymorphism (SSCP) analysis, we have identified mutations in 10 patients . Four of these mutations are single nucleotide insertions. Three of them were present in channel forming region of exon13. Other insertion mutation was found in carboxyterminal region of exon21 . All three-insertion mutations manifested hepatological problems in early childhood .His 1070 Gln mutation was found in four patients. Ile1103 thr mutation was found in two children. Altogether, this study infers that insertion type of mutations lead to early hepatic manifestation.



**Genetic mapping of Chuvash Polycythemia: An autosomal recessive disorder of oxygen sensing.** *D.W. Stockton<sup>1</sup>, H. Chen<sup>1</sup>, S.O. Ang<sup>1</sup>, A.I. Sergeyeva<sup>2</sup>, V.R. Gordeuk<sup>3</sup>, L.A. Polyakova<sup>2</sup>, J.T. Prchal<sup>1</sup>.* 1) Baylor College of Medicine, Houston, TX; 2) Chuvash State University, Cheboksary, Chuvashia, Russian Federation; 3) Howard University, Washington, DC.

**Purpose:** Familial erythrocytosis (MIM #263400) or Chuvash polycythemia (CP) is a recessive disorder of increased red cell mass endemic to the mid-Volga River region of Russia. In addition to high hematocrits (60%-70%) the condition manifests cardiovascular disease at an early age and possibly increased risk for malignancy. CP patients exhibit both indicators of primary and secondary polycythemias. We hypothesize a founder effect mutation in the hypoxia sensing pathway may play a role in its pathogenesis. Hypoxia inducible factor 1a (HIF-1a), is a major regulator of hypoxia inducible genes. The locus for Familial Benign Polycythemia (FBP) in Chuvash families was previously reported in chromosome band 11q23. Combining these hypotheses a functional and genetic approach was undertaken to identify the causal gene. **Methods:** We reviewed the clinical phenotype and the response of erythroid precursors to EPO, measured HIF-1a expression in lymphocytes cultured with or without cobalt, and measured total iron binding capacity and serum concentrations of iron, ferritin, and circulating transferrin receptor (TfR). Six families were used for linkage analysis between the CP phenotype and the HIF-1a and reported FBP locus genomic regions. A genome screen was started to localize the responsible gene. **Results:** TfR levels were inappropriately increased. Normoxic HIF-1a protein and mRNA levels were elevated and after exposure to cobalt, HIF-1a mRNA levels did not change in CP cells while they increased 5-fold in controls. Linkage analysis between the CP phenotype and the HIF-1a and the reported FBP loci resulted in LOD scores less than -2 across the regions. Preliminary positive LOD scores have been identified. **Conclusions:** Chuvash polycythemia is an autosomal recessive erythrocytosis caused by abnormalities of oxygen sensing. A major regulator of hypoxic adaptation, HIF-1a and the previously reported locus were genetically excluded. Additional candidate genomic regions have been identified that require verification.

**Mutations of the P and MC1R genes produce OCA2 with red hair.** *R.M. Schmidt<sup>1</sup>, J.E. Pietsch<sup>1</sup>, J.P. Fryer<sup>1</sup>, C.G. Summers<sup>2</sup>, W.S. Oetting<sup>1</sup>, R.A. King<sup>1</sup>.* 1) Medicine/Inst of Human Genetics, University of Minnesota, Minneapolis, MN; 2) Ophthalmology, University of Minnesota.

**Background:** P gene mutations produce oculocutaneous albinism type 2 (OCA2), characterized by the presence of pigmented yellow/blond or brown hair throughout life. We have identified an individual with oculocutaneous albinism who has had bright red hair since birth; red hair was present in both maternal and paternal relatives. The skin was white and did not tan. Irides were blue, nystagmus and an alternative strabismus were present, and best corrected visual acuity was 20/60. We hypothesized that this phenotype was the result of mutations at two genes, one responsible for the albinism (P gene) and one responsible for the red hair (MC1R). **Methods:** Sequence analysis of the P and MC1R genes was performed with DNA from the proband and parents using direct automated infrared fluorescence sequencing. Sequence analysis included all coding exons and the intron:exon borders. **Results:** The proband was a compound heterozygote for two mutations of the P gene - N489D and W679C; both of these mutations have previously been identified in individuals with OCA2. In addition, the proband was heterozygous for the R160W variant of the MC1R gene; this polymorphism has been associated with red hair. **Conclusion:** The pigment phenotype of OCA2 is broad, and usually is thought to include yellow/blond to brown hair. We have now identified an individual with OCA2 who has red hair, associated with P gene mutations and an additional change in the MC1R gene. The phenotype results from changes in two genes that influence pigment formation. This is the first demonstration of a molecular mechanism for OCA associated with red hair. Furthermore, this type of OCA should be differentiated from OCA3 or rufous/red OCA that is associated with a different phenotype and gene.

**The MYHIIA syndrome: A novel spectrum of autosomal dominant macrothrombocytopenias.** *K.E. Heath<sup>1</sup>, A. Campos-Barros<sup>1,2</sup>, A. Toren<sup>3</sup>, G. Rozenfeld-Granot<sup>3</sup>, L.E. Carlson<sup>4</sup>, J. Savage<sup>5</sup>, J.C. Denison<sup>6</sup>, M.C. Gregory<sup>6</sup>, J.A. White<sup>7</sup>, D.F. Barker<sup>6</sup>, A. Greinacher<sup>4</sup>, C.J. Epstein<sup>8</sup>, M.J. Glucksman<sup>1</sup>, J.A. Martignetti<sup>1</sup>.* 1) Dept. of Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) University Hospital Niño Jesus, Madrid, Spain; 3) The Chaim Sheba Medical Center, Tel-Hashomer, Israel; 4) Ernst-Moritz-Amdt University of Greifswald, Germany; 5) Austin and Repatriation Medical Center, University of Melbourne, Australia; 6) University of Utah School of Medicine, UT; 7) University of Minnesota, MN; 8) University of California, San Francisco, CA.

May-Hegglin anomaly (MHA), Fechtner (FTNS) and Sebastian (SBS) syndromes form a group of autosomal dominant disorders that share macrothrombocytopenia (MTCP) and leukocyte inclusions. FTNS has the additional features of nephritis, deafness and cataracts. Previously, we identified that mutations in the nonmuscle myosin heavy chain 9 gene (*MYH9*), encoding MYHIIA, resulted in all three disorders. We have now expanded our studies to examine the spectrum of mutations, genotype:phenotype relationships, and structure-function correlates in a large cohort of patients including individuals with two other related platelet syndromes: Epstein (EPS) and Alport-like with MTCP (APSM). We now demonstrate that EPS and APSM are also caused by *MYH9* mutations. In total, 8 different mutations were identified in 20 affected individuals (74%). The R702C and R702H mutations were only found in FTNS, EPS and APSM which all share MTCP, nephritis and deafness; suggesting that they represent variants of the same syndrome. Moreover, these results define a critical region of MYHIIA that seems to be critical in the pathogenesis of nephritis and deafness. The coiled-coil domain mutations, E1841K, D1424N and R1933X, were common to both MHA and FTNS. A common haplotype was shared by three E1841K carriers (1 MHA, 2 FTNS). This suggests both a common ancestor and a potential disease modifier gene. Altogether, our data suggest that these 5 syndromes define a broad phenotypic spectrum of disorders all caused by *MYH9* mutations. We therefore propose the MYHIIA syndrome to encompass these disorders. (E-mail: kheath@hgmp.mrc.ac.uk).

**Cellular localization of mRNA expression of Usher IIa gene in rodent retina.** *D. Huang, D. Pretto, C. Talmadge, G. Zhou, M. Zhou, J.R. Davis, J. Eudy, E. Uzvolgyi, J. Sumegi.* Pathology and Microbiology, Center for Human Molecular Genetics, University of Nebraska Medical Center, Omaha, NE, USA.

Usher Syndrome (USH) is a clinically and genetically heterogeneous autosomal recessive disorder comprising hearing and visual impairment. It has been subdivided into 3 clinical subtypes, Usher type I (USHI), type II (USHII), and type III (USHIII). At present, 10 distinct loci have been identified for the 3 clinical subtypes. Mutations in Usher IIa gene account for the most common clinical type of Usher syndrome, USHII, which has the phenotype of moderate to severe hearing impairment, normal vestibular function, and later onset of retinal degeneration. The goal of our research is to identify the cellular source of the Usher IIa mRNA in developing and adult rodent retina. We isolated the rat and the mouse homologues of Usher IIa gene. Digoxigenin labeled RNA was synthesized in vitro from the cloned cDNA fragments and used in situ hybridization. We found that Usher IIa mRNA expression appeared to be restricted to photoreceptor cell outer nuclear layer in adult rat and mouse retina. In developing rat retina, Usher IIa mRNA can be detected as early as embryonic day 17 in the developing neural layer of the retina. RT-PCR showed that Y79, a human photoreceptor specific cell line expresses the Usher IIa transcript while the rat retina pigment epithelial cell line, RPEJ does not. This data is consistent with the results obtained by in situ hybridization.

**Molecular characterization of the 22q13 deletion syndrome: comparison of over 50 cases.** *H.E. McDermid<sup>1</sup>, W.Y. Tse<sup>1</sup>, G.A. Stapleton<sup>2</sup>, L. Artifoni<sup>3</sup>, B. Dallapiccola<sup>4</sup>, H.L. Wilson<sup>1</sup>*. 1) Dept Biological Sciences, Univ Alberta, Edmonton, AB, Canada; 2) Greenwood Genetic Center, Greenville, SC; 3) Università di Padova, Padova, Italy; 4) CSS-Mendel Institute, University La Sapienza, Rome, Italy.

Patients with the 22q13 deletion syndrome show profound global developmental delay, absent/severely delayed expressive speech, hypotonia, normal to excessive growth and mild dysmorphic features (Phelan et al, 2001). A critical region for at least a subset of neurological abnormalities associated with this syndrome has been suggested based on a patient, NT (Flint et al, 1995). NT has a 130 kb terminal microdeletion and shows developmental delay and severe delay of expressive speech. In order to confirm this critical region, we have delineated the deletion size in over 50 additional 22q13 deletion syndrome patients using microsatellite analysis and fluorescence in situ hybridization. In all but one of the patients, the deletion was terminal. These deletions ranged from less than 1 Mb to approximately 11 Mb, however the major neurological features of developmental delay and delay of speech were present in all of these cases. This confirms that a major candidate gene for this syndrome maps to the most distal Mb of 22q, and adds support for our hypothesis that this candidate gene is SHANK3 (formerly PROSAP2), which codes for a structural protein of the post-synaptic density and which localizes to the 130 kb NT deletion.

A single patient has an interstitial deletion, which does not overlap with the 15 smallest terminal deletions, but may fall entirely within some of the largest deletions. This patient, who therefore has a different but overlapping syndrome, may be helpful in identifying other genes affecting only the larger deletion cases.

**Instability of normal sized FMR1 alleles in two generations: evidence for a modifier effect?** *D. Kennedy, T. Ashfield, J. Guo, C. Pereira, D. Ramadeen, R. Rezaie, J. Steer, J. Tzountzouris, D.J. Allingham-Hawkins.* Molecular Genetics, North York General Hosp, North York, ON., Canada.

Fragile X syndrome is caused by the expansion of an unstable trinucleotide repeat, CGGn, in the 5' untranslated region of the FMR1 gene located at Xq27.3. Affected individuals generally have CGG>200. Alleles in the normal range, CGG6-54, are generally considered to be stably transmitted while those in the premutation range, CGG52-200, tend to change in size upon transmission to offspring. We recently reported a group of developmentally delayed males who demonstrated apparent instability of FMR1 alleles in the normal range (Tzountzouris et al., 2000). This population of patients represents approximately 1% of the patients tested for Fragile X syndrome in our laboratory. Here we present a family in which both a developmentally delayed boy and his father demonstrate two FMR1 alleles in the normal range upon PCR analysis. Neither of the child's alleles corresponds to a paternal allele while the presence of one maternal allele can be demonstrated. Paternity testing is pending. The presence of Y chromosome material was demonstrated in both the father and child by PCR analysis of the amelogenin locus. Southern blot analysis on the child's FMR1 gene showed the presence of the 2.8 kb band only suggesting that an inactive X chromosome is not present. Karyotype analysis on the child revealed a normal 46,XY karyotype on all 30 cells analysed, ruling out the possibility of Klinefelter's syndrome or mosaic Klinefelter's syndrome. Karyotype and Southern blot analyses on the father are pending. This family is interesting because it is the first time that instability of normal FMR1 alleles is apparent in both a parent and child. There are a number of possible explanations: a cryptic chromosome rearrangement involving the Xq27.3 band, non-paternity or, most interestingly, a modifier gene on an autosome or the Y chromosome that is contributing to instability of the FMR1 repeat. Molecular and cytogenetic analyses are ongoing to help elucidate the mechanism of instability in this family.

**Novel Molecular Mechanism for Occipital Horn Syndrome.** *S.G. Kaler<sup>1</sup>, P.-C. Liu<sup>1</sup>, D.J. Wattendorf<sup>2</sup>, K.N. Rosenbaum<sup>3</sup>*. 1) Clinical Neurosciences Program, Natl Inst Neurologic Disorders & Stroke, NIH; 2) Med Genet Branch, NHGRI, NIH Bethesda, MD; 3) Dept. Medical Genetics, Children's National Med Ctr, Washington, DC.

Occipital horn syndrome (OHS) is a mild allelic variant of Menkes disease (MD), an X-linked disorder of copper transport. Whereas MD combines severe neurological problems with connective tissue abnormalities including skin laxity, bladder diverticula and vascular tortuosity, OHS patients have minimal neurological problems. The molecular basis of OHS typically involves splice junction mutations that either reduce proper mRNA splicing, or lead to mislocalization of the gene product. One example of a deletion in the 5' regulatory region has also been reported. Here we report the first example of a missense mutation associated with OHS, in a male who is also the youngest (13 months) patient with this phenotype described to date.

The proband had a normal birth and was well until 9 months of age when urinary obstruction due to bladder diverticula was noted. Additional clinical findings including cutis laxa and coarse hair led to consideration of OHS at 13 months of age. Blood copper (98 mg/dl) and ceruloplasmin (331 mg/L) were well within the normal ranges for age, however plasma neurochemical levels indicated deficiency of dopamine beta hydroxylase (DBH), a copper-dependent enzyme. Sequence analysis of the MD/OHS locus disclosed a novel missense mutation (G892D) in exon 13.

This is the first example of a missense mutation causing OHS. The presence of normal circulating copper levels (without copper therapy) and absence of significant neurological findings implies that this mutation does not impair copper exodus at the GI tract or blood-brain barrier. However, the abnormal neurochemical pattern and connective tissue findings are consistent with deficient activities of DBH and lysyl oxidase, cuproenzymes processed within the trans-Golgi apparatus. These cumulative findings suggest that G892D induces mislocalization of the Menkes/OHS gene product, an hypothesis testable by immunocytochemical or *in situ*PCR experiments using this patient's cells.

**Characterization of a Subclass of Patients with Ehlers-Danlos Syndrome Type VI that Appears Unrelated to a Decrease in One of the Lysyl Hydroxylase Isoforms.** *H.N. Yeowell<sup>1</sup>, L.C. Walker<sup>1</sup>, M. Willing<sup>2</sup>, J.C. Marini<sup>3</sup>, W.A. Cabral<sup>3</sup>, E. Kitamura<sup>4</sup>, M. Yamauchi<sup>4</sup>.* 1) Div of Dermatology, Duke Univ Medical Ctr, Durham, NC; 2) Dept of Pediatrics, Univ of Iowa, Iowa City, IA; 3) Heritable Disorders Branch, NICHD, Bethesda, MD; 4) Dental Research Center, Univ. of N. Carolina, Chapel Hill, NC.

Ehlers-Danlos syndrome (EDS) type VI is an autosomal recessive disorder in which patients are characterized clinically by kyphoscoliosis, hypermobile joints, and skin that is fragile and hyperextensible and, biochemically, by a deficiency of the enzyme lysyl hydroxylase 1 (LH1, PLOD1). LH1 hydroxylates specific lysine residues in the collagen molecule that are critical in determining the pathways of intermolecular crosslinks essential for the tensile strength of collagen. A subclass of EDS VI exists in which patients have certain clinical characteristics of EDS VI but their levels of LH activity are normal. To examine the biochemical basis for this subtype, we compared levels of the mRNAs for LH1 and the two other isoforms for LH, LH2 and LH3, with the pattern of crosslinking, and enzyme activity in fibroblasts from 10 patients. In contrast to the typical EDS VI patients, in which levels of LH1 mRNA and corresponding LH activity are <25% of control, LH1 mRNA levels in this subgroup of patients ranged between 87-150% of control which correlated with their normal LH activity. However, decreased levels of LH2 mRNA were observed in two patients (45 and 54% of control, respectively), and in the first patient, the low (45%) LH2 mRNA was accompanied by a sharper decrease (16% of control) in the mRNA for LH3. Another patient also had decreased levels of LH3 mRNA (35% of control). Studies on long-term cultures of fibroblasts did not show a correlation between levels of the collagen reducible crosslinks, dehydrodihydroxylysinonorleucine and dehydrohydroxylysinonorleucine, and levels of mRNA for the LH isoforms. This study suggests that, unless this group of patients represents a heterogeneous subset of this EDS VI variant or an unidentified LH isoform is involved, an alternative pathway other than lysine hydroxylation of collagen may be affected.



**Cryptic splicing at a non-consensus splice-donor-site in a patient with a new mutation in the plakophilin 1 gene.**

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Plakophilin 1 (*PKP1*) is a structural component of the desmosomes, which are intercellular adhesion junctions primarily found in epithelial cells. Mutations in the *PKP1* gene result in skin fragility ectodermal dysplasia syndrome, an autosomal recessive disease affecting skin, nails and hair. We identified a new homozygous mutation in the exon 9 splice-donor-site of *PKP1* (IVS9+1 G>A) in a patient with a relatively mild phenotype of erosive skin lesions, palmoplantar hyperkeratosis and loose hair. Transcriptional analysis of *PKP1* from a skin biopsy demonstrates complete functional inactivation of the mutated splice-donor-site. Besides transcripts in which intron 9 is retained, we observe alternate spliced transcripts in which a cryptic splice-site within exon 9 is actively used. Interestingly, this cryptic splice-donor-site does not contain a consensus GT but instead uses a GC, which has not been reported before in man. In addition, this alternate splicing preserves the correct open reading frame, putatively removing only 15 amino acids from the mature PKP1 protein. The functional rescue of the major part of the PKP1 protein by the alternate pre-mRNA splicing may explain the mild phenotype observed in this particular patient compared to the previously described patients. This unique genotype may eventually give new insights in the functional interactions of the PKP1 protein with other proteins of the desmosome.

**FOXL2 Mutations in type I and type II Blepharophimosis/Ptosis and Epicanthus Inversus Syndrome (BPES).** *M. Uda*<sup>1</sup>, *L. Crisponi*<sup>2</sup>, *M. Deiana*<sup>1</sup>, *F. Chiappe*<sup>2</sup>, *G. Usala*<sup>1</sup>, *P. Amati*<sup>3</sup>, *D. Bonneau*<sup>3</sup>, *F. Faravelli*<sup>4</sup>, *J. Tolmie*<sup>5</sup>, *L. Bisceglia*<sup>6</sup>, *L. Zelante*<sup>6</sup>, *A. Iolascon*<sup>7</sup>, *P. Gasparini*<sup>6</sup>, *G. Cripioni*<sup>2</sup>, *A. Cao*<sup>1</sup>, *G. Pilia*<sup>1</sup>. 1) IRTAM, CNR, Cagliari, Cagliari, Italy; 2) University of Cagliari, Italy; 3) Service de Genetique Medicale, Poitiers, France; 4) Ospedale Galliera, Genova, Italy; 5) Yorkhill NHS Trust, Glasgow, UK; 6) IRCCS, Ospedale CSS, San Giovanni Rotondo, Italy; 7) University of Bari, Bari, Italy.

In Type I Blepharophimosis/Ptosis/Epicanthus inversus Syndrome (BPES) eyelid abnormalities are associated with Ovarian Failure leading to Female Infertility. Apparently, type II shows only the eyelid defects and affected female could transmit the disorder. We have recently cloned a novel, winged helix/forkhead transcription factor gene, FOXL2, that we found to be mutated in both types of BPES. We report here on a FOXL2 mutation screening we carried out on 35 BPES families. Nonsense and frameshift mutations were identified on all 13 type I BPES families we analyzed. In 6 type II BPES patients we observed apparently milder mutations: a 30 bp in-frame duplication (909\_939dup) present in 3 independent type II BPES families, 2 different missense mutations and a 3 basepair deletion of the last codon, leading to a protein one amino acid-shorter than normal. Also we detected FOXL2 mutation in 7 additional BPES patients whose clinical type could not be determined. We could not find mutations in 4 type II and in 5 BPES families of unknown type. Mutations outside the FOXL2 gene or mutations in another gene could be responsible for these cases. These results further support the notion that FOXL2 loss-of-function mutations are probably incompatible with any kind of female fertility. Milder mutations could allow a child-bearing activity in affected females, but these mutations could nonetheless affect ovarian function and female reproductive life-span in type II BPES. In one instance for example, the 909\_939dup was present in a 14 years old girl with overt Ovarian Failure whose affected mother also had a history of Premature Ovarian Failure. These data therefore suggest that all female BPES patients with FOXL2 mutations should be carefully evaluated for their ovarian function.

**Mouse containing a human chromosome 21 models Downs syndrome.** *M. Oshimura<sup>1</sup>, T. Shinohara<sup>1</sup>, K. Tomizuka<sup>2</sup>, S. Miyabara<sup>3</sup>, Y. Kazuki<sup>1</sup>, S. Ikegami<sup>4</sup>, K. Inokuchi<sup>4</sup>, I. Ishida<sup>2</sup>.* 1) Dept Molecular Cell Genetics, Tottori Univ Sch Life Sci, Yonago, Japan/CREST, JST, Japan; 2) Pharmaceutical Res Lab, KIRIN Brewery Co. Ltd, Takasaki, Japan; 3) Dept Pathology, Saga Medical School, Saga, Japan; 4) Mitsubishi Kasei Inst Life Sci, Machida, Japan.

Trisomy 21(TS21) results in a constellation of features known as Downs syndrome (DS). To investigate the gene dosage effects of an extra copy of human chromosome 21 (Chr 21) on various phenotypes, we used microcell-mediated chromosome transfer to create mouse embryonic stem (ES) cells containing Chr 21. ES cell lines retaining Chr 21 as an independent chromosome were used to produce chimeric mice with a substantial contribution from Chr 21-containing cells. Fluorescence *in situ* hybridization and PCR-based DNA analysis revealed that Chr 21 was substantially intact but had sustained a small deletion. The freely segregating Chr 21 was lost during development in some tissues. These chimeric mice showed a high correlation between retention of Chr 21 in the brain and impairment in learning or emotional behavior. Hypoplastic thymus and cardiac defects were observed in a considerable number of chimeric mouse fetuses with a high contribution of Chr 21. However, each chimeric mouse has a different pattern of trisomic and euploid cells, complicating analyses. Thus, we are developing mice that have Chr 21 and defined fragments translocated to a mouse chromosome, using the Cre-loxP chromosome engineering techniques.

**C7orf2 gene is interrupted within intronic region by a de novo reciprocal chromosomal translocation t (5, 7)(q11, q36) in a patient with preaxial polydactyly.** *T. Horikoshi*<sup>1,2</sup>, *N. Endo*<sup>2</sup>, *M. Shibata*<sup>3</sup>, *M. Suzuki*<sup>4</sup>, *E. Takahashi*<sup>4</sup>, *T. Shinka*<sup>5</sup>, *Y. Nakahori*<sup>5</sup>, *D. Ayusawa*<sup>6</sup>, *K. Nakabayashi*<sup>7</sup>, *S.W. Scherer*<sup>7</sup>, *S. Noji*<sup>2</sup>. 1) Department of Orthopedic Surgery, Niigata University, Niigata, Japan; 2) Department of Biological Science and Technology, Faculty of Engineering, University of Tokushima, Tokushima, Japan; 3) Division of Plastic & Reconstructive Surgery, School of Medicine, University of Niigata, Japan; 4) Otsuka GEN Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan; 5) Department of Public Health, School of Medicine, University of Tokushima, Tokushima, Japan; 6) Kihara Institute for Biological Research, Graduate School of Integrated Science, Yokohama City University, Yokohama, Japan; 7) Department of Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Canada.

Human preaxial polydactyly (PPD) is an autosomal dominant developmental disorder, and its locus has been mapped to chromosome 7q36. We performed fine mapping of the 7q36 breakpoint of a de novo reciprocal translocation t (5, 7)(q11, q36) in a patient with sporadic PPD. FISH analysis and genomic Southern blot showed that C7orf2 gene, which is located in the vicinity of SONIC HEDGEHOG (SHH), was directly interrupted by the translocation breakpoint within intronic region. C7orf2, which encodes a putative transmembrane protein, has recently been identified as causative gene for a rare autosomal recessive congenital malformation of the limb, acheiropodia. However, no mutation within the coding sequence of C7orf2 has been found in PPD families. Another candidate gene, SHH, has been known to cause preaxial polydactyly when it is misexpressed in the anterior limb bud. SHH encodes a signaling molecule, which is thought to determine antero-posterior axis of the limb. Taken together, we speculate the two possible underlying genetic mechanisms for the mutation. First, truncated C7orf2 gene product generated by translocation may function as a dominant active form. Second, a distant limb-specific regulatory element of SHH may exist within C7orf2 genic region. To clarify which is right, genetic studies using animals and further genomic survey in PPD families remain to be done.

**Analysis of *ROR1* gene in a patient with *dirdup(1)(p21p31)* and clinical signs of Robinow syndrome.** *J.F. Mazzeu*<sup>1</sup>, *Z. Mustacchi*<sup>2</sup>, *R.C.M. Pavanello*<sup>1</sup>, *A.M. Vianna-Morgante*<sup>1</sup>. 1) Department of Biology, Universidade de São Paulo, São Paulo, Brazil; 2) Department of Genetics, Universidade Paulista, São Paulo, Brazil.

Robinow syndrome is characterized by fetal facies, orodental abnormalities, mesomelic dwarfism, and hypoplastic genitalia. A dominant and a recessive form of this syndrome have been described and can be distinguished by the severe mesomelic dwarfism and multiple rib and vertebral anomalies present in the recessive type. The mutated gene responsible for the recessive form has been mapped to 9q22 and then identified as *ROR2* gene. The *ROR2* protein is 58% homologous to *ROR1* protein, and *ROR1* maps to chromosome 1p31. Patients with duplications involving 1p31 have clinical features that resemble those of the dominant form of Robinow syndrome. A deletion of this segment has been already reported in a patient with the diagnosis of Robinow syndrome. Herein we describe a girl with clinical signs of Robinow syndrome, who carries a partial duplication of the short arm of chromosome 1: 46,XX,*dirdup(1)(p21p31)* after G banding. The association of Robinow syndrome features and chromosome alterations involving the segment 1p31, where *ROR1* maps, led us to investigate this gene as a candidate for the dominant form of Robinow syndrome. To refine the breakpoint analysis we performed FISH of sequences mapped to 1p as well as microsatellite genotyping. The proximal breakpoint could be restricted to the segment between YACs 943\_f\_11 and 784\_b\_5 at 1p21 and 1p13, respectively. The distal breakpoint was found to be located between YACs 892\_h\_12 and 842\_b\_8 at 1p31, thus coinciding with the mapping site of the *ROR1* gene. The hybridization of the *ROR1* intragenic probe AI206250 (Incyte Genomics), after Southern blotting of genomic DNA *EcoR* 1 digests, revealed a fragment of 4,4kb in the patient, her parents and two normal controls. A less intense extra fragment of 7.3 kb was also present in the patient, pointing to the disruption of *ROR1* gene. These results suggest *ROR1* gene as a candidate for the dominant form of Robinow syndrome.

**Sorting nexin 3 (SNX3) is disrupted in a patient with a translocation t(6;13)(q21;q12) and Microcephaly, Microphthalmia, Ectrodactyly, Prognathism (MMEP) phenotype.** V.S. Vervoort<sup>1,2</sup>, D. Viljoen<sup>3</sup>, R. Smart<sup>3</sup>, G. Suthers<sup>4</sup>, B. Dupont<sup>1</sup>, A. Abbott<sup>2</sup>, C. Schwartz<sup>1,2</sup>. 1) Greenwood Genetic Center, Greenwood, South Carolina; 2) Genetics and Biochemistry, Clemson University, Clemson, SC; 3) Department of Human Genetics, SAIMR, Johannesburg, South Africa; 4) Women's & Children's Hospital, North Adelaide, Australia.

MMEP is a rare disorder characterized by microcephaly, microphthalmia, ectrodactyly and prognathism. A patient with MMEP and mental retardation was previously reported to carry a *de novo* reciprocal t(6;13)(q21;q12) translocation (Viljoen and Smart, 1993). In an attempt to identify the presumed causative gene, we mapped the translocation breakpoints using Fluorescent *in situ* Hybridization (FISH). Two overlapping genomic clones crossed the breakpoint on the der(6) chromosome, locating the breakpoint region between D6S1594 and D6S1250. This region contains the Sorting Nexin 3 (SNX3) gene. Southern analysis allowed us to characterize the der(6) breakpoint and determine that SNX3 was disrupted. Using inverse-PCR, we were able to amplify and sequence the der(6) breakpoint region. The sequence exhibited homology to a BAC clone that contained marker D13S250. This allowed us to amplify and sequence the der(13) breakpoint region and to determine that no additional rearrangement was present at either breakpoint, nor was another gene disrupted. Therefore, the translocation was balanced and SNX3 was determined to be a candidate gene for MMEP. However, mutation screening by SSCP and Southern analysis of a sporadic case with MMEP (Suthers and Morris, 1996) failed to detect any point mutations or deletions in the SNX3 coding sequence. Considering the possibility of positional effect, another candidate gene could map in the vicinity of the der(6) chromosome breakpoint. Alternatively, another locus not on chromosome 6 may cause MMEP in the second patient. Several rearrangements involving the 6q21 region have been associated with ectrodactyly and various somatic malformations, which may be allelic forms of the MMEP phenotype. Therefore, SNX3 or other genes in the same region could be candidates for mutation screening in sporadic cases with ectrodactyly.

**A 1.5 million base pair inversion polymorphism in families with Williams-Beuren syndrome.** *L.R. Osborne<sup>1</sup>, M. Li<sup>2</sup>, B. Pober<sup>3</sup>, D. Chitayat<sup>2</sup>, J. Bodurtha<sup>4</sup>, T. Costa<sup>5</sup>, T. Grebe<sup>6</sup>, S. Cox<sup>6</sup>, L.C. Tsui<sup>2</sup>, S.W. Scherer<sup>2</sup>.* 1) Dept Medicine, Univ Toronto, Toronto, ON, Canada; 2) Dept Genetics, Hospital for Sick Children, Toronto, ON, Canada; 3) Dept Genetics, Yale University School of Medicine, New Haven, CT; 4) Dept Human Genetics, Virginia Commonwealth University, Richmond, VA; 5) Dept Pediatrics, Atlantic Research Centre, Halifax, NS, Canada; 6) Genetics Program, University of Arizona, Phoenix AZ.

Williams-Beuren syndrome (WBS) is caused by hemizygous deletion of a 1.5 Mb interval encompassing more than 23 genes at 7q11.23. The mechanism underlying the deletion is unequal meiotic recombination mediated by highly homologous DNA flanking the commonly deleted region. These flanking duplicons contain transcribed genes and conserved pseudogenes and are comprised of blocks of near identical DNA (>95% identity) occurring in the same and also opposite orientations. We hypothesized that in addition to the deletions commonly seen in WBS, the duplicons may mediate other genomic rearrangements. Using interphase FISH we identified a genomic polymorphism in WBS families consisting of an inversion of the entire WBS region. The inversion was found hemizygously in 3 of 11 (27%) atypical patients whom exhibit a subset of the WBS phenotypic spectrum and do not carry the typical WBS microdeletion. Moreover, in 4 of 12 (33%) families examined with a deleted WBS proband, the inversion was observed exclusively on the disease-transmitting parental chromosome. One atypical individual with the inversion polymorphism and a t(6;7)(q27;q11.23) through the ELN gene enabled a detailed analysis of the WBS region with more than 20 FISH probes from within the deletion region and surrounding chromosomal segment, and showed that the inversion breakpoints lie within the duplicons. These results suggest this newly identified genomic variant can be associated directly with atypical forms of the disease, as well as predispose to primarily WBS-causing microdeletions, but also translocations and inversions. Our findings support the idea that genomic polymorphism might be an important contributor in other *de novo* chromosome rearrangements and disease previously thought to be stochastic in nature.

**Histone Hypoacetylation of Subtelomeric DNA Repeats (D4Z4) Whose Deletion Causes Fascioscapulohumeral Muscular Dystrophy.** *G. Jiang, F. Tsien, M. Ehrlich.* Human Genetics, Tulane Cancer Center, Tulane Medical School, New Orleans, LA.

Fascioscapulohumeral muscular dystrophy (FSHD) is an unusual autosomal dominant syndrome caused by the loss of some copies of a complex repeat (D4Z4) in the subtelomeric region of one chromosome 4 homologue. The number of copies of this 3.3-kb repeat at 4q35 arm is polymorphic. Unaffected individuals have 11 to about 95 copies on each 4q35 while > 90% of FSHD patients have <10 copies at one 4q35. Many investigators have proposed that normally this region is heterochromatic but that when the number of tandem copies of D4Z4 is <10, the region loses its condensed chromatin structure. This is hypothesized to induce inappropriate gene expression in the affected muscle cells. However, there have been no reports of the chromatin structure in this region. Because constitutive heterochromatin has hypoacetylated core histones, which lead to chromatin compaction, we are testing the acetylation of D4Z4 repeat chromatin using chromatin immunoprecipitation (ChIP) with an antibody for tetra-acetylated histone H4 followed by PCR with various amounts of immunoprecipitated sample for DNA amplification. We compared H4 acetylation of chromatin containing the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene, D4Z4 repeats, or centromeric satellite a repeats by ChIP assays on a normal lymphoblastoid cell line. *GAPDH* served as a euchromatic standard and satellite a repeats as the heterochromatic standard. H4 acetylation was quantitated from the immunoprecipitated chromatin normalizing for the PCR efficiency. The relative amounts of H4 acetylation were 1.0, 0.01, and <0.01, for *GAPDH*, D4Z4, and satellite a, respectively, which is consistent with heterochromatinization of D4Z4. We will analyze chromatin containing D4Z4, satellite a, or various euchromatic genes for mono- and tetra-acetylation of histone H4 to determine if D4Z4 chromatin is normally hypoacetylated compared to bulk euchromatin, as is satellite a heterochromatin. We will also compare cells from FSHD patients and analogous cells from unaffected individuals to determine if the D4Z4 repeats from the deletion-containing 4q35 are abnormally hyperacetylated in FSHD cells. Supported by FSH Society Grant FSHS-MB-06.



**Presence of a double homeobox gene in the D4Z4 locus of a patient with FSHD despite a subtelomeric exchange between chromosomes 4q35 and 10q26.** *I. Leclercq<sup>1</sup>, C. Mattéotti<sup>1</sup>, G. Deneubourg<sup>1</sup>, S.M. Van der Maarel<sup>2</sup>, R.R. Frants<sup>2</sup>, G. Padberg<sup>3</sup>, F. Coppée<sup>1</sup>, A. Belayew<sup>1</sup>.* 1) Lab. Molecular Biology, University of Mons-Hainaut, Mons, Belgium; 2) MGC Dept Human Genetics, Leiden University Medical Center, Leiden, The Netherlands; 3) Dept Neurology, University of Nijmegen, Nijmegen, The Netherlands.

The 4q35 D4Z4 locus comprises 10-100 tandem copies of a 3.3 kb repeat associated with heterochromatin. Heterozygous deletions leaving 1-10 repeats in D4Z4 are linked to facioscapulohumeral muscular dystrophy (FSHD). We have identified an intronless gene encoding a protein with a double homeodomain (DUX4) in each of the two 3.3 kb repeats left in the altered D4Z4 locus of a patient with FSHD (Gabriëls et al, 1999, Gene 236: 25-32). *DUX4* was functional as shown by transfection experiments, and the encoded protein located to the nuclear envelope. We hypothesize that partial D4Z4 deletions seen in patients could destabilize heterochromatin, allowing expression in some cells of the *DUX4* gene present in the few 3.3 kb repeats left. The resulting protein would be toxic to muscles. The D4Z4 repeat array has a homologue on chromosome 10q26. An argument raised against our hypothesis is the occurrence of subtelomeric exchanges between chromosomes 4 and 10, which are sometimes observed in patients. These can replace some D4Z4 repeats by D10Z10 ones (Lemmers et al, 1998, Hum Mol Genet 7: 1207-1214). We analysed genomic DNA from a patient with FSHD who had only two 3.3 kb repeats in his affected 4q35 locus, both originating from chromosome 10. One 3.3 kb unit was subcloned and sequenced, showing the presence of a *DUX10* gene very similar to *DUX4*. In precisely comparing *DUX4* and *DUX10*, we found sequencing errors in the very GC rich *DUX4* 3' end: their correction yielded an ORF encoding an identical DUX4/10 double homeodomain protein. In conclusion, our hypothesis on the role of a *DUX* gene in FSHD is still valid. It would also predict a specific feature on chromosome 4 driving *DUX* expression, since short repeat arrays on chromosome 10 are not pathogenic. (Supported by AFM-France, FSH Society, and MDA).

**Molecular genetic study of three patients with the 22q11 deletion syndrome.** *F. Rahkhoodae<sup>1</sup>, B. Dion<sup>1</sup>, P. Kleinfinger<sup>2</sup>, A. Aurias<sup>2</sup>, S. Demczuk<sup>1</sup>.* 1) Research Institute , Montreal Children's Hospital , Montreal, Quebec, Canada; 2) Institut Curie Section Recherche, Paris, France.

Microdeletions of chromosome 22 are associated with many developmental defects with a broad spectrum of clinical features such as hypocalcemia, heart defect, cleft palate, facial dysmorphism, and mental retardation. Patients vary in the size of the missing chromosomal region, but most of them have a 3 million bp interstitial deletion. Others have very small deletions or other type of rearrangements and very few patients have a normal chromosomal set with no apparent rearrangements. Many efforts have been developed to delimit a critical region for this syndrome. From these studies, it appears that there are at least two critical regions, one in the proximal part of the deleted region and one in the distal part, from which a number of genes have been isolated.

Three patients with typical phenotype have been studied, and preliminary analysis by FISH and FIBER-FISH strongly indicate the presence of a deletion in the 22q11 region. Cell lines from each patient were established. In order to separate the maternal and paternal (or normal vs deleted) chromosomes, somatic cell hybrids were constructed. The hybrid cell lines were verified for the integrity of the 22q11 chromosomal region. Equally spaced primer pairs were chosen from the cosmid suspected to be deleted in each patient and PCR was performed to confirm the existence of the deletion. For two of the three patients, this experiment did not confirm the presence of the deletion. The last patient is being studied. Nonetheless, these patients are invaluable tools to study the molecular pathogenesis of this syndrome. Specific genes for which there is recent evidence for an involvement in the syndrome (TBX1, CRKL, UFD1L) will be examined for point mutations and rearrangements in these patients.

Finding the responsible gene or genes for the 22q11 deletion syndrome will have an impact in the accurate diagnosis of the disease and might open the door to prognostic predictions which will be especially useful in prenatal diagnosis situation.

**Heterozygous PITX2/RIEG1 gene deletion associated with GH deficiency in Rieger syndrome.** *P. Bitoun<sup>1</sup>, K. Machinis<sup>2</sup>, E. Semina<sup>3</sup>, M. Jesuran-Perelroizen<sup>4</sup>, M. Feingold<sup>5</sup>, S. Odent<sup>6</sup>, B. Le Marec<sup>6</sup>, F. Dastot<sup>2</sup>, J. Gaudelus<sup>1</sup>, J.C. Murray<sup>3</sup>, S. Amselem<sup>2</sup>, V. Cacheux<sup>2</sup>.* 1) Medical Genetics and Pediatrics, CHU Paris-Nord, Hopital Jean Verdier, 93143 Bondy, France; 2) Cytogenetics and Molecular Genetics, Hopital Henri Mondor, Creteil,; 3) Dept of Genetics and Biological Sciences, U of Iowa, Iowa City Ia; 4) 15 rue du rempart Saint-etienne, Toulouse; 5) National Birth Defects Center, Waltham Ma; 6) Medical Genetics, Hopital Pontchaillou, Rennes.

The gooseoid homeobox transcription factor gene PITX2/RIEG1 is involved with Rieger syndrome which associates Rieger ocular anomaly, umbilical defects and oligodontia. Pitx2 also plays a key role in left-right axis patterning of heart, gut and lungs. Approximately 44% of our 30 patients with Rieger syndrome were found to have mutations in the PITX2 gene at 4q25. None of the families with short stature and associated growth hormone deficiency were found to have mutations within the coding region or splice sites of the PITX2 gene. Purpose : Because PITX2 is strongly expressed in midline structures like dental primordia, umbilicus and early in the pituitary anlage in mice and because of its role in left-right axis patterning PITX2 deletion could be involved with the GH deficiency of the Rieger syndrome. Patients and Methods : Lymphocytes, fibroblast cell lines and DNA samples from Patients with Rieger syndrome or various forms of selected anterior segment anomalies associated with GH deficiency were tested for heterozygous PITX2 gene deletion. The 4 cosmid clones identified to clone the PITX2 gene were used as fluorescent probes for hybridisation to the patients chromosomes from lymphocyte or fibroblast cell lines using FISH techniques. DNA samples were also obtained to test for hemizygosity at the PITX2 locus using microsatellite markers. Results : We found a heterozygous PITX2 deletion spanning the entire gene in one of two families with Rieger syndrome and GH deficiency. Only DNA being obtained from the other families, deletion will be tested by genotyping microsatellite markers. Our data are consistent with a major role of the PITX2 gene in human pituitary development.

**A novel mutation in RDS/Peripherin gene causing butterfly-shaped pattern dystrophy and adult onset foveal macular dystrophy.** Z. Yang<sup>1</sup>, Z. Yu<sup>1</sup>, Y. Li<sup>1</sup>, S. Jacobson<sup>2</sup>, S. Thirumalaichary<sup>1</sup>, D. Zack<sup>3</sup>, K. Zhang<sup>1</sup>. 1) Cole Eye Inst, Cleveland Clinic Foundation, Cleveland, OH; 2) University of Pennsylvania, Philadelphia, PA; 3) Wilmer institute, John Hopkins School of Medicine, Baltimore, MD.

**Purpose:** Butterfly-shaped pattern dystrophy (BPD) and adult onset foveal macular dystrophy (AOFMD) are two similar forms of autosomal dominant macular dystrophy characterized by deposits in the retinal pigment epithelium. Patients with these diseases present with pigmentary macular lesions with reduced visual acuity in the fifth decade. Various mutations in the RDS/peripherin gene, which encodes a photoreceptor specific glycoprotein, have been associated with both conditions. We identified one BPD family and one AOFMD family and conducted clinical and genetic analyses. **Methods:** Ophthalmologic examination and fluorescein angiography were performed in members of these two families. Mutational screening was performed by direct sequencing of PCR-amplified DNA fragments corresponding to the three exons of the gene. **Results:** Of the 5 patients at risk for BPD and 13 patients at risk for AOFMD in two Caucasian kindreds, four and eight individuals demonstrated macular changes on ophthalmoscopic examination and fluorescein angiography characteristic of BPD and AOFMD respectively. The BPD phenotype in the first family is relatively consistent in all four patients. However, there is a marked variation in AOFMD phenotype in the second family, ranging from mild pigmentary changes in the fovea, to macular geographic atrophy or choroidal neovascularization. Sequencing analysis identified an A to G change at nucleotide 422 predicting a novel Tyr141Cys substitution in both families. This change occurred in a highly conserved Tyrosine residual and segregated with the disease phenotype in both families. This change did not occur in 200 control chromosomes. **Conclusion:** We identified a novel mutation in the RDS/peripherin gene in two independent families. This mutation appears to cause diverse clinical phenotypes, such as BPD or AOFMD. Characterization of full spectrum of mutations in the RDS/peripherin gene will provide useful diagnostic and prognostic information.

**Mutation screening and further analysis of *TBX1* in non-deleted patients with DiGeorge/Velocardiofacial syndrome (DGS/VCFS).** W. Gong<sup>1</sup>, S. Gottlieb<sup>1</sup>, J. Collins<sup>1</sup>, A. Blescia<sup>1</sup>, H. Dietz<sup>2</sup>, D.M. McDonald\_McGinn<sup>1</sup>, E.H. Zackai<sup>1</sup>, B.S. Emanuel<sup>1</sup>, D.A. Driscoll<sup>1</sup>, M.L. Budarf<sup>1</sup>. 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Howard Hughes Medical Institute, John Hopkins University School of Medicine, Baltimore.

Recent studies using murine models suggest that *TBX1* is a candidate gene for DGS/VCFS (Jerome and Papaioannou 2001, Lindsay et al., 2001, Merscher et al., 2001). *TBX1* is a member of T-box gene family and haploinsufficiency of T-box genes, *TBX3* and *TBX5* are associated with human genetic diseases. To better understand the role of *TBX1* in the etiology of DGS/VCFS, we performed mutation screening of *TBX1* in 40 patients with the diagnosis of DGS/VCFS but without a detectable 22q11 deletion. PCR was performed with genomic DNA from patients and normal controls. SSCP and/or CSGE were used to screen for mutations of *TBX1*. Heterozygous changes were confirmed by direct sequencing. We identified five rare variants, including one single base change (-39C/T), one 3-bp insertion and three deletions (del 8 bp, del 9 bp, del 15 bp) that were not observed in over 100 normal controls. Sequence analysis of the family members of these patients demonstrates that these rare variants were transmitted from an unaffected parent to proband. We are performing in vitro assays to determine whether the rare variants of *TBX1* are diseases-causally related to DGS/VCFS. Among the rare variants detected, four of the five rare variants are located in the last exon and the 8-bp deletion is predicted to cause a frame shift at amino acid 425. We have generated cDNA constructs containing the full-length wild-type *TBX1*, the 8-bp deletion variant *TBX1* and a partial *TBX1* cDNA sequence without the terminal exon. These constructs allow us to analyze the expression and subcellular localization of the normal and variant forms of *TBX1* protein in the HEK 293 cells and Cos7 cells by immunofluorescence and immunoblotting. The characterization of the normal and altered forms of *TBX1* proteins will help us to understand the function of *TBX1* protein.

***de novo* mutation in the *SIX6* homeobox gene in a patient with bilateral anophthalmia.** M.E. Gallardo<sup>1,2</sup>, A.S. Schneider<sup>3</sup>, M.A. Dwyer<sup>3</sup>, S. Rodríguez de Córdoba<sup>1,2</sup>. 1) Unidad de Patología Molecular, Fundación Jiménez Díaz, Madrid, Spain; 2) Dept Immunología, CIB (CSIC), Madrid, Spain; 3) Dept Genetics, Albert Einstein Medical Ctr, Philadelphia, PA.

*SIX6* is a recently characterized member of the *SIX/sine oculis* family of homeobox genes. *SIX6* is expressed in the developing and adult retina, in the optic nerve and in the hypothalamic and pituitary regions. *SIX6* maps to chromosome 14q22.3-q23 within a region that is deleted in three individuals with bilateral anophthalmia and pituitary anomalies. To determine the implication of *SIX6* in ophthalmological malformations we have screened 30 families from the Anophthalmia /Microphthalmia Registry at Albert Einstein Medical Center, Philadelphia for mutations in *SIX6*. We have identified a patient who carries a mutation (c.59C®T) in the heterozygous form in the 5 untranslated region of the *SIX6* gene. This is the first time that a mutation in *SIX6* has been observed. The patient is a 7 year old female with bilateral anophthalmia born to consanguineous parents. Her karyotype was normal. Growth and development are appropriate for age and she has no known anomalies. The mutation is not present in the parents. Correct segregation of the parental chromosomes in the patient was demonstrated using four polymorphic markers (D14S997, D14S1038, D14S1010 and D14S1007) in the *SIX6* region. Since c.59C®T is clearly a *de novo* mutation in *SIX6* in the anophthalmic patient, it would be expected that this mutation is responsible for the disorder. However, the functional consequences of the c.59C®T mutation are unknown. A number of experiments are being performed to determine whether the c.59C®T mutation results in abnormal expression of the *SIX6* gene.

**Physical and transcriptional map of the Hereditary Inclusion Body Myopathy (HIBM) locus on chromosome 9p12-p13.** *I. Eisenberg<sup>1</sup>, H. Hochner<sup>1</sup>, M. Shemesh<sup>1</sup>, T. Levi<sup>5</sup>, T. Potikha<sup>1</sup>, M. Barash<sup>1</sup>, G. Grabov<sup>1</sup>, M. Sadeh<sup>2</sup>, Z. Argov<sup>3</sup>, C.L. Jackson<sup>4</sup>, S. Mitrani-Rosenbaum<sup>1</sup>.* 1) Molecular Biology Unit, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; 2) Department of Neurology, Wolfson Hospital, Holon, Israel; 3) Department of Neurology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; 4) Department of Pathology, Rhode Island Hospital and Brown University, Providence, Rhode Island, USA; 5) Department of Genetics, Harvard Medical School, Boston, Massachusetts, USA.

Hereditary Inclusion Body Myopathy (HIBM)(OMIM 600737) is a unique group of neuromuscular disorders characterized by adult-onset, slowly progressive distal and proximal muscle weakness and typical muscle pathology. Previously, we have mapped the gene causing the recessive form of HIBM to chromosome 9p1 and narrowed the interval to one single YAC clone of 1Mb in size. As a further step towards the identification of the HIBM gene, we have constructed a detailed physical and transcriptional map of this region. A high resolution BAC contig that includes the HIBM critical region, flanked by marker 327GT4 and D9S1859, was constructed. This contig allowed the precise localization of twenty five genes and ESTs to the proximal region of chromosome 9. The expression pattern of those mapped genes and ESTs was established by Northern blot analysis. In the process of refining the HIBM interval, thirteen new polymorphic markers were identified, of which 11 are CA-repeats, and 2 are single nucleotide polymorphisms. Certainly, this map provides an important integration of physical and transcriptional information corresponding to chromosome 9p12-p13, which is expected to facilitate the cloning and identification not only of the HIBM gene, but also other disease genes which map to this region.

**Association Between Angiotensin-Converting Enzyme and Alzheimer Disease in the Korean population.** *E.S.*

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Angiotensin-Converting Enzyme (ACE) has been reported to show activity in patient with neurologic disease. An insertion-deletion polymorphism in ACE has recently been linked to heart disease, cerebrovascular disease, and Alzheimer Disease (AD). Other findings demonstrated a significantly higher ACE activity in the hippocampus, frontal and temporal cortex and other cerebral regions in subjects with AD. It has been recently shown that the ACE D allele bearing subjects have an increased risk of cognitive impairment, independent of other common risk factors such as advanced age, female gender and presence of Apolipoprotein E (APO-E) \*4 allele. In order to verify the association of ACE gene with AD, as well as its association with APO-E genotype, we performed ACE genotyping in subjects with Late-Onset Alzheimer's disease (LOAD, n=62), 68 healthy age-matched controls and 472 healthy controls, who were previously characterized for APO-E genotype. After the principal component analysis, ACE D and Apo-E \*4 alleles disclosed the highest prevalence in the Alzheimer's disease group in Korea ( $P < 0.05$ ). We concluded that in the population studied here, the ACE D acts in association with the ApoE \*4 as a susceptibility gene for AD.



**The Fanconi Anemia Mutation Database:** <http://www.rockefeller.edu/fanconi/mutate/>. A.D. Auerbach<sup>1</sup>, K. Pujara<sup>1</sup>, S.D. Batish<sup>1</sup>, O. Levrin<sup>1</sup>, C.G. Mathew<sup>2</sup>. 1) Lab Human Gen & Hematology, Rockefeller Univ, New York, NY; 2) Div. Medical & Molecular Genetics, Guy's Hospital, London, UK.

Fanconi anemia (FA) is an autosomal recessive syndrome with diverse clinical features that is characterized by DNA crosslink hypersensitivity and cancer predisposition. Eight complementation groups and 6 unique genes have now been identified (FANCA: OMIM 227650; FANCB: 227660; FANCC: OMIM 227645; FANCD1: OMIM 605724; FANCD2: OMIM 227646; FANCE: OMIM 600901; FANCF: OMIM 603467; FANCG: OMIM 602956). Detailed information regarding all the variants (mutations and polymorphisms) identified in FA genes is maintained in the Fanconi Anemia Mutation Database (FAMd), a model locus specific database (LSDB) for the Mutation Database Initiative (MDI, [www.genomic.unimelb.edu.au/mdi/](http://www.genomic.unimelb.edu.au/mdi/)). The FAMd was established on the worldwide web (<http://www.rockefeller.edu/fanconi/mutate/>) in 1997 and was redesigned and updated in June, 2001. Its goal is to serve researchers in the field by providing a means for rapid republication and dissemination of mutation data. Passwords are currently required for access to the "private" section of the database containing unpublished mutations, and are provided to all individuals who agree to abide by a set of guidelines protecting the priority rights of the researchers who submit data prior to publication. The database is maintained in a Filemaker Pro Database (Claris Corporation) on a Power Macintosh Server. Nomenclature conforms to the guidelines specified by the Nomenclature Working Group of the MDI. A summary of the number of variants in the database for the 6 cloned genes is shown.

	FANCA	FANCC	FANCD2	FANCE	FANCF	FANCG	Total
Variants	201	24	5	3	6	36	275

By contributing data to the future Central Database of MDI, FAMd helps provide investigators and clinicians with universal free access to the assembled total knowledge of human gene variation.

**Human disease genes: Protein function and clinical phenotypes.** *G. Jimenez-Sanchez, B. Childs, D. Valle.* Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

Availability of whole genome sequence from humans and from several model organisms has accelerated the identification of genes responsible for human genetic disease. To search for correlations between the function of the protein products of these "disease genes" and features of the corresponding disease, we assembled a list of nearly 900 human disease genes. We compared the function of their protein products to characteristics of the disease phenotypes such as frequency, mode of inheritance, age of onset, and impact on life expectancy. Originally we analyzed enzymes, modulators of protein function, receptors, and transcription factors (*Nature* 409: 853, 2001) and have currently extended our study to include intracellular structural proteins and extracellular matrix components and by subdividing functional categories that appeared to be correlated with certain aspects of disease phenotype. For example, we found that genes encoding transcription factors (TF) are a major cause of genetic diseases with onset *in utero*: 36 of 61 (59%) TF genes responsible for genetic disease produce phenotypes *in utero*. Classification of TF in TRANSFAC categories (<http://transfac.gbf.de/TRANSFAC/>) showed that 56% of these 36 (20) are members of the helix-loop-helix (HLH) superclass, most of these (12) are in the homeodomain class. By contrast, only 30% of the 23 TF causing disease with onset between 1 and 50 years are in the HLH superclass including only 4 (17%) in the homeodomain class; 9 (39%) are in the Zn finger superclass including 6 (26%) nuclear receptors of the Cys4 Zn finger class. An analysis of the human genome revealed >2000 hypothetical TF genes, with C2H2 Zn finger the largest class among transcriptional activators (*Nature* 409:832, 2001). Only 3 of the 61 TF (5%) causing disease are in this class. Similar differences in age of onset are apparent in genes encoding enzymes, most of which have their onset in extrauterine life. As knowledge of disease genes grows, including those contributing to complex traits, more sophisticated analyses will be possible; their results will yield deeper understanding of disease and enhanced integration of medicine with biology.

**The human LDL receptor gene (LDLR) database: Molecular analysis of 625 mutations.** *L. Villeger<sup>1</sup>, M. Abi-Fadel<sup>1</sup>, S. Rochaud<sup>1</sup>, A. Robin<sup>1</sup>, J-P. Rabes<sup>1,2</sup>, C. Junien<sup>1,2</sup>, C. Beroud<sup>1</sup>, C. Boileau<sup>1,2</sup>, M. Varret<sup>1</sup>.* 1) INSERM U383, Hopital Necker-Enfants Malades, Paris, France; 2) Laboratoire central de biochimie et de genétique moléculaire, CHU Ambroise Paré, Boulogne, France.

To date, 711 mutations have been identified in the LDLR gene, encoding the low-density lipoprotein receptor, in subjects with Familial Hypercholesterolemia. Although genotype/structure-function correlations have been substantially investigated, genotype/phenotype correlations have not been explored. Thus, we have compiled a database containing standardized data for each LDLR mutation, and developed the software that provides sorting tools and allows optimized multicriteria research [<http://www.umd.necker.fr>]. The analysis of the 625 point mutations in the UMD-LDLR database gives the following information: [1] 58% of the mutations are missense, and 17% occur in CpG dinucleotides known to be mutational hot spots; [2] although widely distributed throughout the gene, there is an excess of mutations in exons 4 and 6 (ligand-binding repeats), 7 (EGF-like repeat), and 9 (EGF-precursor-like); [3] there is a deficit of mutations in exons 10 and 13 (EGF-precursor-like), 15 (O-linked-sugar), 16 (transmembrane), 17 and 18 (cytoplasmic); [4] 47% of the small deletions occur between repeated sequences and can be explained by the slipped-mispairing model described by Krawczak and Cooper; [5] 68% of the mutations in the ligand-binding domain affect conserved amino-acids involved in LDL binding; [6] the functional data available for 183 (29%) mutations indicate 38% of class 2B (transport defective) and 33% of class 1 mutations (null alleles); [7] finally, the investigation of genotype/phenotype correlations is difficult since the clinical data is usually incomplete in mutation reports. Direct access to the database through the web site should facilitate the input of high quality clinical information and should overcome this shortage.

**Identification of a novel GUCY2D mutation in an Iranian family with Leber Congenital Amaurosis.** *T. Rezaie<sup>1</sup>, M.H. Karimi-Nejad<sup>2</sup>, M. Meshkat<sup>3</sup>, S. Sohbati<sup>2</sup>, R. Karimi-Nejad<sup>2</sup>, H. Najmabadi<sup>2</sup>, M. Sarfarazi<sup>1</sup>.* 1) Molecular Ophthalmic Genetics, Univ of Connecticut Health Center, Farmington, CT; 2) Karimi-Nejad Pathology & Genetic Center, Tehran, Iran; 3) Department of Ophthalmology, Kerman University of Medical Sciences, Kerman, Iran.

Evaluation of congenital blindness in southeastern region of Iran identified an extended family with Leber Congenital Amaurosis (LCA) in the Lore Tribe of Sirjan, Kerman. Immediate relatives of 8 affected subjects interconnected through 11 different generations shown an autosomal recessive pattern of inheritance. Full clinical and ophthalmological examinations revealed infantile nystagmus, keratoconus, narrowing of retinal vessels, retinal degeneration, mild pigmentary retinopathy and electrophysiological investigations consistent with diagnosis of LCA. DNA samples from first-degree relatives in 5 distantly related branches were used for linkage evaluation to known LCA loci on chromosomes 1, 6, 14, 17 and 19. Only one locus on 17p13.1 was consistent with linkage in 19 subjects of only 4 branches. Inspection of the inherited haplotypes suggested that a mutation in the LCA1/GUCY2D but not the LCA4/AIPL1 is more likely to be involved. Therefore, this gene was amplified and fully sequenced in an affected subject. A homozygous missense mutation (ATC->AGC; I816S) within the exon 13 of GUCY2D was identified. Sequencing of a 328-bp PCR fragment containing this mutation in 19 subjects fully segregated in 6 affected, 8 gene carrier parents and 5 normal members, including 2 heterozygote gene carriers. SSCP screening of I816S did not identify this mutation in 184 normal control chromosomes (70 Iranian and 114 other Caucasians). I816S falls within a putative dimerization domain of the guanylate cyclases protein and it is fully conserved in mouse, rat and bovine. We also identified one wobble (CAC->CAT; H247H) and another polymorphism (CTC->CAC; L782H) in this gene. Identification of I816S mutation provides a rapid molecular diagnosis for over 150 other members of this family that were not included in our initial screening. The I816S is a novel mutation in GUCY2D gene and, this is the first mutation report in an Iranian LCA family.

**Mutation analysis of KIT gene in human piebaldism.** *K. Fukai, N. Oiso, A. Kato, N. Hosomi, T. Murakami, M. Ishii.*  
Dept Dermatology, Osaka City Univ Medical Sch, Osaka, Japan.

Piebaldism is an autosomal dominant disorder, characterized by congenital circumscribed depigmented macules most frequently on the forehead, abdomen, and knees. This disorder has been shown to occur due to germline mutations of the c-KIT gene, which is necessary for the development and the differentiation of dermal melanocytes during embryogenesis. We had the oppertunities to study the c-KIT gene in 17 families with human piebaldism: four from Japan, four from Italy, four from the USA, two from UK, two from Holand, and one form Columbia. Genomic DNAs were prepared from peripheral leukocytes, and all exons and flanking introns were PCR-amplified, and direct-sequenced. We were able to identify mutations in 7 families. Among these, three were frameshift mutations, 142delG, 2247-2250delAAGA, 1768-1769delAG, and three missense mutations, C136R, D792Y, and V620A, and one nonsense mutation, E346X. All mutations except C146R were novel and not reported in the literature. We are screening SCF (stem cell factor) gene for KIT-mutation-negative cases with piebaldism.

**Molecular evidence for a founder effect in South African Afrikaners with pseudoxanthoma elasticum.** *K.B. Beck<sup>1</sup>, O. Le Saux<sup>1</sup>, C. Sachsinger<sup>1</sup>, C. Treiber<sup>1</sup>, AS. Marais<sup>2</sup>, E. Johnson<sup>3</sup>, L. Bercovitch<sup>4</sup>, S.F Terry<sup>5</sup>, D.L. Viljoen<sup>6</sup>, C.D. Boyd<sup>1</sup>.* 1) Pacific Biomed Research Ctr, University of Hawaii, Honolulu, HI; 2) Department of Genetics University of Cape Town Medical School; 3) Barrow Neurological Institute, Phoenix, AZ; 4) Department of Dermatology, Brown Medical school, Providence, RI; 5) PXE International Inc., Sharon, MA; 6) South African Inst. for Med. Res., University of Witswatersrand, Johannesburg, South Africa.

Pseudoxanthoma elasticum (PXE) is a heritable disorder characterized by dermal, vascular and ocular lesions resulting from the mineralization of elastic fibers in these tissues. Recently, the first mutations responsible for the development of PXE have been identified. The observed prevalence of this disorder is approximately the same in all population groups studied with one exception among South African Afrikaners. Previous reports suggested founder effects as a possible explanation for a higher prevalence of PXE in Afrikaners, a Europeans-derived population that first settled in South Africa in the 17th century. To investigate this hypothesis, we have performed a mutational analysis of ABCC6 in 17 apparently unrelated Afrikaner families with PXE, in addition to 6 unrelated PXE families from South Africa of British and Indian descent. In the Afrikaner population, 6 different mutations were characterized. Three alleles were missense variants, 2 were nonsense and 1 was a single base pair insertion. One mutation, R1339C, has been found to occur at a frequency of 53; others were noted at lower frequencies ranging from 3 to 12%. Putative disease-causing mutations were identified in about 85% of the 34 chromosomes studied. A single R1339C was found in a heterozygous state in a control panel of 100 alleles derived from unaffected Afrikaner individuals. In the 6 families of different origins, the mutations detection rate was 75%. Four out-of-frame insertion or deletions, 2 nonsense mutations and 1 missense variant were identified. Haplotype analysis of the Afrikaner families revealed that the 3 most frequent mutations were identical-by-descent, indicating a founder origin of PXE in this population.

**PAX3 and MITF Mutations in Colombian Patients with Waardenburg Syndrome.** *N.Y. Gelvez<sup>1</sup>, M. Rodriguez<sup>1</sup>, M.C. Lattig<sup>1</sup>, J.C. Prieto<sup>1</sup>, T. Friedman<sup>2</sup>, R. Morell<sup>2</sup>, M.L. Tamayo<sup>1</sup>.* 1) Instituto Genetica Humana, Universidad Javeriana, Bogota, Colombia; 2) NIDCD, NIH, Bethesda, MA, USA.

During the past two years we performed a screening program for Waardenburg Syndrome (WS) in Colombia, finding a frequency of 5.3% of the syndrome among the deaf institutionalized population. We evaluated 93 families (120 affected individuals) with clinical characteristics of WS. Twenty-seven families (27/93) were classified as WS type 1 (29%), fifty-six (56/93) as WS type 2 (60.2%) and ten (10/93) as no-determinate WS type (10.8%). We performed SSCPs for PAX 3 (responsible for WS1) and MITF (responsible for WS2) in the total of 93 detected families. All affected families were tested for both genes. The general results of the molecular analysis were: For PAX 3 gene we found 19 families with any mutation (20.3%), and for MITF gene we found mutation in 15 families (16%). The detailed results by each gene, were: PAX3 gene mutations in exon 2: 12.9% (12/93); exon 4: 1.07% (1/93); exon 5: 1.07% (1/93); exon 6: 2.1% (2/93) and exon 7: 3.2,% (3/93). For the MITF gene, we found mutation in exon 1: 2.1% (2/93) and exon 9: 13.9% (13/93). Sequence analysis is in progress to confirm the type of mutation. Results by type of syndrome were more interesting. We discuss the use of the molecular study to define the diagnosis in WS patients with no-determinate clinical type. Our population has a bigger frequency for WS type 2 (60,2%) than WS type 1, opposite to previous reports. Although we have not yet analyzed the exons 9 and 10 for the PAX 3 gene, the heterogeneity of the syndrome is clear due to the fact that 75,2% of the total of our affected population did not present mutation in any of the known causing genes.

**Mal de Meleda (MDM) is caused by mutations in the SLURP-1 gene in patients from Palestine and the United Arab Emirates.** *K.M. Eckl<sup>1,2</sup>, H.P. Stevens<sup>3</sup>, G.G. Lestringant<sup>4</sup>, P.M. Frossard<sup>4</sup>, I.M. Leigh<sup>3</sup>, P. Nürnberg<sup>1</sup>, A. Reis<sup>5</sup>, H.C. Hennies<sup>1</sup>.* 1) Molecular Genetics and Gene Mapping Center, Max Delbrück Center, Berlin, Germany; 2) Faculty of Biology, Chemistry, and Pharmacy, Free University, Berlin, Germany; 3) Center for Cutaneous Research, St Bartholomew's and the Royal London School of Medicine, London, UK; 4) Tawam Hospital and Faculty of Medicine and Health Sciences, Al Ain, United Arab Emirates; 5) Human Genetics, Friedrich Alexander University, Erlangen, Germany.

Mal de Meleda (MDM), or keratosis palmoplantaris transgrediens of Siemens, was first reported on the island of Meleda, now Mljet, in Croatia. It is characterized by diffuse palmoplantar keratoderma (PPK) and transgressive keratosis with an onset in early infancy before one year of age. There is no associated involvement of other organs. A rather broad spectrum of clinical presentations with other optional and variable features is characteristic of MDM. Some patients show marked progression of most symptoms of the disease. A locus for MDM was found on chromosome 8q24-qter, and recently, mutations in the *ARS (component B)* gene were identified in patients with MDM. However, we have shown lately that a very similar phenotype of transgressive PPK is not linked to the interval on chromosome 8q in several families from the United Arab Emirates. Here we analyzed further families with transgressive PPK. In a large Palestinian pedigree with multiple consanguinity, patients were homozygous for a new mutation, 1764G>A, in *ARS (component B)*, which substitutes an arginine for a conserved glycine residue. Moreover, another new mutation, 578A>C, was seen in patients from an Emirati family, which alters the translation initiation codon. *ARS (component B)* encodes the secreted Ly-6/uPAR-related protein-1 (SLURP-1). SLURP-1 is a member of the Ly-6 protein superfamily and belongs to a subgroup that also contains various secreted cytotoxins. These findings show that the MDM type of transgressive PPK may be caused by SLURP-1 mutations also in other than Croatian patients. A founder effect is supposed in patients from Mljet, however, allelic heterogeneity was observed here for mutations in the SLURP-1 gene.



**A spectrum of ABCC6 mutations is responsible for pseudoxanthoma elasticum.** *O. Le Saux*<sup>1</sup>, *K. Beck*<sup>1</sup>, *C. Sachsinger*<sup>1</sup>, *C. Silvestri*<sup>1,2</sup>, *C. Treiber*<sup>1</sup>, *H. Gring*<sup>3</sup>, *E. Johnson*<sup>4</sup>, *A. De Paepe*<sup>5</sup>, *M. Pope*<sup>6</sup>, *I. Pasquali-Ronchetti*<sup>2</sup>, *L. Bercovitch*<sup>7</sup>, *S.F. Terry*<sup>8</sup>, *C. Boyd*<sup>1</sup>. 1) Pacific Biomed Research Ctr, University of Hawaii, Honolulu, HI; 2) Department of Biomedical Sciences, University of Modena, Modena, Italy; 3) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 4) Barrow Neurological Institute, Phoenix, AZ; 5) Center for Medical Genetics, University Hospital, Universitair Ziekenhuis Gent; Belgium; 6) MRC Connective Tissue Genetics Group, Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK; 7) Department of Dermatology, Brown Medical School, Providence, RI; 8) PXE International Inc., Sharon, MA.

To better understand the pathogenetics of pseudoxanthoma elasticum (PXE), we have performed a mutational analysis of ABCC6 in 122 unrelated PXE patients, the largest cohort of patients yet studied. Thirty-six mutations were characterized and 26 were novel variants. 21 alleles were missense variants, 6 were small insertion or deletions, 5 were nonsense, 2 were likely to result in aberrant mRNA splicing and 2 were large deletions involving ABCC6. The total number of ABCC6 mutations known to date is 43. Whilst most mutations appeared to be private variants, two disease-causing alleles occurred frequently in apparently unrelated individuals. R1141X was found at a frequency of 18.8% and was preponderant in European patients. ABCC6del23-29 occurred at a frequency of 12.9% and was prevalent in patients from the United States. These results suggested that R1141X and ABCC6del23-29 might have been derived regionally from founder alleles. Putative disease-causing mutations were identified in 64% of the 244 disease alleles studied. Our results suggest that the undetected mutant alleles could either be genomic rearrangements or mutations occurring in non-coding regions of the ABCC6 gene. The distribution of ABCC6 mutations revealed a cluster of disease-causing variants within exons encoding a large C-terminal cytoplasmic loop and in the C-terminal nucleotide-binding domain (NBD2). We discussed the structural and functional significance of this mutation pattern.

**Survival of male cases of incontinentia pigmenti carrying a lethal mutation can be explained by somatic mosaicism or a 47,XXY karyotype.** S.J. Kenwick<sup>1</sup>, A. Smahi<sup>2</sup>, S. Aradhya<sup>3</sup>, G. Shuttleworth<sup>4</sup>, S. Lyonnet<sup>2</sup>, C. Kashork<sup>3</sup>, H. Woffendin<sup>1</sup>, E. Mayer<sup>4</sup>, L. Greenhalgh<sup>5</sup>, T. Jakins<sup>1</sup>, M. Levy<sup>3</sup>, R. Lewis<sup>3</sup>, L. Shaffer<sup>3</sup>, A. Munnich<sup>2</sup>, D. Nelson<sup>3</sup>. 1) WTCMMD, Univ. Cambridge, Cambridge, U.K; 2) Hôpital Necker-enfants Malades, Paris, France; 3) Baylor College of Medicine, TX, USA; 4) Bristol Eye Hospital, U.K; 5) St Michael's Hospital, Bristol, U.K.

incontinentia pigmenti (IP) is an X-linked dominant disorder characterised by abnormalities of skin, teeth, hair and eyes, skewed X-inactivation and recurrent male miscarriages. IP results from mutations in the gene for NEMO with deletion of exons 4-10, mediated by tandem repeats, accounting for ~80% of new mutations. Males inheriting this mutation and other null mutations die *in utero*. Less deleterious mutations give rise to surviving males with ectodermal dysplasia and immunodeficiency and female carriers with variable or no IP. Males with abnormalities typical of IP (without immunodeficiency) are rare and have been found with a 47,XXY karyotype. However, 46,XY cases have also been reported. We describe three 46,XY and one 47,XXY male cases of IP who presented with all the hallmarks of IP (neonatal blistering, keratotic lesions, hyperpigmentation, dermal scarring and hypoplastic teeth). PCR and Southern blot analysis demonstrates that the 47,XXY male has inherited the common deletion from his IP-affected mother, and an additional X chromosome from his father. He has selectively eliminated cells expressing the mutated X resulting in skewed X-inactivation. The 46,XY cases also have both wild type and deleted copies of *NEMO* and are therefore mosaic. Furthermore, analysis of samples from a 9yr old IP boy shows that cells expressing the mutated X are selectively eliminated. Thus, the repeat-mediated recombination leading to the common deletion is not restricted to meiosis and most likely occurs via intrachromosomal exchange. Post-zygotic deletion may explain reports of anticipation in IP pedigrees. Finally, karyotypic abnormalities and post-zygotic mutation provide two mechanisms for survival of males carrying a *NEMO* mutation.

**Anhidrotic Ectodermal Dysplasia with Immunodeficiency not associated with NEMO mutations.** A. Smahi<sup>1</sup>, G. Courtois<sup>3</sup>, R. Doffinger<sup>2</sup>, J.L. Casanova<sup>2</sup>, A. Munnich<sup>1</sup>. 1) Unite de Recherches sur les Handicaps Genetiques de l'Enfant. INSERM U393, Hopital Necker, Paris, France; 2) Laboratoire de Genetique Humaine des Maladies Infectieuses, Faculte de Mdecine Necker-Enfants Malades, Paris, France; 3) URA CNRS 1773, Institut Pasteur, Paris, France.

Anhidrotic Ectodermal Dysplasia (EDA) is a rare developmental syndrome responsible for absent sweat glands, sparse scalp hair and rare conical teeth. Mutations in the Ectodermal Dysplasia 1 (ED1) and downless (dl) genes account for a form of X-linked recessive and autosomal recessive/dominant type of EDA respectively. On the other hand, EDA with Immunodeficiency is a X-linked recessive condition which associates ectodermal dysplasia with impaired response to various pathogens (EDA-ID). This syndrome has been recently ascribed to hypomorphic mutations in the NEMO gene, resulting in impaired NF-KB signaling. Here we describe two EDA-ID syndrome patients with no mutation of the NEMO gene. Patient 1, the son of a healthy mother, had mild EDA and was highly susceptible to infectious disease caused by micro-organisms including Gram positive Cocci, Gram negative bacilli and mycobacteria. Bone marrow transplantation was performed at the age of 2 years. The second patient, a girl with Ectrodactyly-ectodermal dysplasia with immunodeficiency, had random X-inactivation. The complete coding region of NEMO gene and the three non coding exons of 5' part were sequenced at the genomic level. No mutation was observed. Mutations in the regulatory regions of NEMO are unlikely as Western blot analysis of patient 1 fibroblasts showed a level of NEMO protein similar to controls fibroblasts. Since a defective IKBalpha degradation after TNFalpha and IL1 stimulation is observed in patient 1 fibroblast, impaired NF-KB activation is most likely. This study suggests that beside NEMO, other autosomal or X-linked recessive genes of the NF-KB cascade can cause EDA-ID in our patients.

**Identification of a Novel Splice Site PPOX Gene Mutation in an Ancient Variegate Porphyria Balearic Family.** *S. Puig<sup>1</sup>, C. Badenas<sup>2</sup>, L. Jimenez<sup>2</sup>, A. Toll<sup>1</sup>, C. Herrero<sup>1</sup>, M. Lecha<sup>1</sup>, M. Mila<sup>2</sup>.* 1) Dermatology Service, Hospital Clinic, Barcelona, Spain; 2) Genetics Service, Hospital Clinic, Barcelona, Spain.

Variegata porphyria (VP) is an autosomal dominant disorder with low penetrance characterised by acute neuro-visceral attacks and skin lesions that can occur separately. The molecular basis of the disorder is a partial deficiency of protoporphyrinogen oxidase encoded by the PPOX gene. This infrequent disease is relatively common in South Africa, where most patients share the same mutation and a common ancestor. Recently, it has been reported the PPOX gene molecular analysis of 108 unrelated VP patients from United Kingdom and France. This study demonstrates VP allelic heterogeneity outside South Africa, and shows that genotype is not a significant determinant of mode of presentation. We have biochemically analysed ninety-six members belonging to a VP family from Mallorca Isle, with 9 symptomatic patients. DNA samples from affected members were screened for mutations in PPOX gene by SSCP analysis. Abnormal pattern in exon 6 was sequenced and a T to A transversion was identified at the consensus splice site (IVS6+2T>A). This change should affect the correct splicing of exon 6 and be the causative mutation in this family. This mutation has not been previously reported, but another exon 6 splice site mutation of PPOX gene has been associated with VP.

**A Tandem Duplication within the Elastin Gene is Associated with Autosomal Dominant Cutis Laxa.** Z. Urban<sup>1</sup>, J. Gao<sup>2</sup>, F.M. Pope<sup>3</sup>, E.C. Davis<sup>2</sup>. 1) Pacific Biomedical Res Ctr, Univ Hawaii, Honolulu, HI; 2) Cell Biology, UT Southwestern Med Ctr, Dallas, TX; 3) Medical Genetics, Univ Hosp Wales, Cardiff, Wales.

Autosomal dominant cutis laxa (adCL) is characterized by redundant, inelastic skin, hernias and, in some cases, pulmonary emphysema and vascular tortuosity. We have used metabolic labeling and immunoprecipitation to analyze elastin gene (ELN) expression in skin fibroblasts from patients with adCL. In addition to the normal 70kD protein, an abnormal 120kD tropoelastin was detected in an adCL patient and her affected daughter. Northern blot analysis confirmed the existence of a correspondingly larger (5 kb instead of 3.5 kb) mRNA. 3'-RACE amplification and RT-PCR experiments indicated that the mutant transcript had the following structure: exons 1-33, 9-33, 9-10, intron 10. Further RT-PCR experiments demonstrated that the normal alternative splicing patterns of exons 23 and 32 were not affected by this duplication. Genomic PCR and DNA sequencing confirmed the duplication breakpoints to be between introns 33 and 8 and Southern-blot experiments demonstrated a tandem duplication at the ELN locus. While the exact pathomechanism of this mutation is not clear, the fact that fibroblasts secrete some of the abnormal tropoelastin suggests a dominant negative effect. Interestingly, a similar tandem duplication in fibrillin-1, another elastic fiber component, causes the tight skin (tsk) phenotype in mice, an apparent opposite of cutis laxa.

**The new mutation Q167K in PIT-1 gene leads to combined pituitary hormone deficiency in an Italian patient.** S. Malvagia, G.M. Poggi, E. Pasquini, M.A. Donati, I. Pela, A. Morrone, E. Zammarchi. Dep. of Paediatrics, Azienda Ospedaliera "A. Meyer" University of Florence, Italy.

Pit-1 gene encodes a transcription factor, important for the development and differentiation of the cells which produce growth hormone (GH), prolactin (Pr1) and thyroid-stimulating-hormone (TSH) in the anterior pituitary gland. Patients with the Pit-1 mutations show a combined pituitary hormone deficiency (CPHD). Eleven mutations have been reported in the Pit-1 gene, up to now. These genetic lesions can be inherited either in an autosomal dominant or an autosomal recessive mode. We report the first Italian patient, a girl, affected by CPHD. At neonatal screening the patient was found positive for congenital hypothyroidism (with low TSH levels). Substitutive therapy was started, but subsequent growth was very poor, while psychomotory development was substantially normal. At the age of 10 months she was hospitalised because of a hypotonic crisis. Clinical examination on admission showed prominent forehead, a depressed nasal bridge, antverted nostrils and a small facial skull. She showed growth retardation (below the 3rd percentile) and her bone age was delayed. EEG was normal. A remarkable nonketotic hypoinsulinemic hypoglycemia (36 mg/dl) was detected with normal cortisol but prolactin was not detectable; IGF-1 and IGFBP-3 were below the normal range. HGH stimulation tests (arginine, clonidine and GHRF) showed a total unresponsiveness. Substitutive hGH therapy (0,6 UI/day) was begun and subsequently the patient gained 2,5 cm/month. Mutation DNA analysis of all 6 exons and exon/intron boundaries in the patients Pit-1 gene identified the novel Q167K amino acid change at the heterozygous level. The highly conserved Q167 residue is located in the POU-specific domain. No mutation was detected in the other allele. DNA analysis in the probands parents did not identify this amino acid substitution, suggesting a de novo genetic lesion. A benign polymorphism for the Q167K amino acids substitution was excluded by restriction enzymatic analysis of 200 normal alleles. Based on these data it can be hypothesised that the Q167K mutation plays a dominant negative effect.

**Mutation in the IgII domain of FGFR2 causes the eponymous form of Pfeiffer syndrome.** *S-H. Kan<sup>1</sup>, N. Elanko<sup>1</sup>, S.R.F. Twigg<sup>1</sup>, L.R. Cornejo-Roldan<sup>2</sup>, M. Muenke<sup>2</sup>, A.O.M. Wilkie<sup>1</sup>*. 1) Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK; 2) Medical Genetics Branch, NHGRI, Bethesda, MD.

In 1964 RA Pfeiffer described a three-generation family showing dominant inheritance of a new form of acrocephalosyndactyly, characterized by craniosynostosis with brachycephaly, broad, medially deviated thumbs and great toes, and variable cutaneous syndactyly. The eponymous label of Pfeiffer syndrome came into use once further patients with this spectrum of features were recognized. In 1994 it was demonstrated that some patients with Pfeiffer syndrome harbour a specific mutation in the fibroblast growth factor receptor 1 (*FGFR1*) gene; the following year, mutations in *FGFR2* were also identified. All *FGFR2* mutations described to date in Pfeiffer syndrome localize to the extracellular IgIII domain.

Investigation of Pfeiffer's original family by Schell *et al* (*Hum Mol Genet* 4:323-328; 1995) showed suggestive linkage to *FGFR2* but did not identify a causative mutation. We revisited this problem by undertaking mutation screening of the entire *FGFR2* gene using Wave DHPLC analysis. We identified substitutions of two adjacent nucleotides, 514G>T and 515C>T, predicting the amino acid substitution A172F, in all three affected members of the family who were available for testing. This substitution replaces a key alanine residue in the IgII domain that is involved in formation of the immunoglobulin fold and makes a receptor:receptor contact during dimerization.

This is the first mutation of the IgII domain to be described in any FGFR, suggesting that further scrutiny of this domain for mutations would be worthwhile. Evaluation of the clinical features described in the original report highlights the relative severity of the limb anomalies in this family.

**MOLECULAR ANALYSIS OF THE CBP GENE IN 65 PATIENTS WITH RUBINSTEIN-TAYBI**

**SYNDROME.** *D. Lacombe*<sup>1,2</sup>, *I. Coupry*<sup>2</sup>, *C. Roudaut*<sup>2</sup>, *M. Steff*<sup>2</sup>, *M.A. Delrue*<sup>1</sup>, *L. Taine*<sup>1</sup>, *B. Arveiler*<sup>1,2</sup>. 1) Dept Medical Genetics, Pellegrin Hospital, Bordeaux, France; 2) University Victor Segalen Bordeaux 2, Bordeaux, France.

Rubinstein-Taybi syndrome (RTS) is a well-known MCA/MR syndrome characterized by growth and mental deficiencies, typical facial dysmorphism, broad thumbs and halluces, various malformations, and an increased risk of neoplasia. RTS is caused by an autosomal dominant mutation, occurring as a submicroscopic deletion or a point mutation, and is due to haploinsufficiency of the CBP (CREB-Binding Protein) gene at chromosome 16p13.3. Gross chromosomal rearrangements and microdeletions detected by fluorescence in situ hybridization (FISH), truncating mutations revealed by the protein truncation test (PTT) and Western blot analysis account for only 20 % of RTS cases. We have developed new molecular tools to thoroughly analyse the CBP gene in a cohort of 65 RTS patients. These include cDNA probes to search for gross rearrangements by Southern blot analysis and to identify CBP mRNA of abnormal sizes on Northern blot, intragenic microsatellite markers to look for intragenic deletions, as well as a complete series of primers to PCR amplify each of the 31 exons of the gene for mutation search by direct sequencing. We have analysed 65 patients using the various techniques and identified 28 mutations : 3 gross rearrangements by Southern blot and/or microsatellite analysis, 1 truncated RNA by Northern blot and 24 point mutations resulting in either stop codons, aminoacid substitutions or abnormal splicing of the CBP RNA. Three additional patients were found to be deleted by FISH analysis. These results showed that a combination of the various techniques allowed to identify a CBP mutation in 47.7 % of RTS cases.



**Sporadic mutations in *TCOF1* arise both in maternal and paternal chromosomes and are not related to advanced parental age.** A. Splendore<sup>1</sup>, E.W. Jabs<sup>2</sup>, M.R. Passos-Bueno<sup>1</sup>. 1) Dept Biol, Univ de Sao Paulo, Sao Paulo, SP, Brazil; 2) Johns Hopkins University School of Medicine, MD, USA.

Sporadic cases of a number of autosomal dominant disorders, including Achondroplasia, Apert, Crouzon and Pfeiffer syndromes have been attributed to advanced paternal age. In all cases, new mutations in the *FGFR* genes that cause these syndromes have been shown to arise exclusively on the paternally-derived chromosome. It has been suggested that new mutations causing Treacher Collins syndrome may also occur preferentially on the germline of older men. Mutations in the *TCOF1* gene are spread throughout its coding region, but exons 23 and 24 are responsible for roughly 1/3 of all known pathogenic changes. To determine whether sporadic mutations in Treacher Collins syndrome occur preferentially on the paternal germline, we selected nine families that had a *de novo* pathogenic mutation either in exon 23 or 24. For all families, paternity was confirmed through five microsatellite markers. A known polymorphism in exon 23 (3938 C®T) was informative for 2 of 4 families with the pathogenic mutation in exon 23. Determination of which polymorphic allele was in *cis* with the mutation was achieved through observation of SSCP patterns and sequencing of PCR products. Through the screening of intron 24, we identified two novel changes (4209+350 C®G and 4209+439 C®A) that allowed us to discriminate paternal and maternal alleles in 4 of 5 families with a 5-bp deletion in exon 24 through ARMS amplification. Three pathogenic mutations - 2 in exon 23 and 1 in exon 24 - were located on the paternally-derived chromosome (mean age 29.5 yrs) and another 3 (all in exon 24) arose on the maternal germline (mean age 25.1 yrs), whereas the 3 remaining families were uninformative. In conclusion, new mutations in *TCOF1*, unlike those in the *FGFR* genes, do not arise preferentially in the paternal germline with no apparent increased age effect. It should be noted that all *FGFR* mutations tested to date were missense, whereas mutations tested in *TCOF1* are frameshift-causing insertions or deletions. This could indicate that these types of change arise through different mechanisms. FAPESP, HHMI, CNPq.

**NOVEL MUTATION IN THE EIF2AK3 GENE IN A CASE OF WOLCOTT-RALLISON SYNDROME. A.**

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The Wolcott-Rallison syndrome (WRS) is an autosomal recessive disorder characterized by permanent neonatal or early infancy insulin-dependent diabetes mellitus, epiphyseal dysplasia and growth retardation. Mutations in the EIF2AK3 gene have been found in 2 families affected by WRS. Eukaryotic Initiation Factor 2a-kinase 3 regulates protein synthesis during stress by phosphorylating the  $\alpha$  subunit of the eukaryotic initiation factor 2. A two months old girl, the only child of non-consanguineous parents, came to our attention because of severe hypertonic dehydration and diabetic ketoacidosis. She had two prolonged convulsive episodes prior to insulin treatment. The family history revealed that the mother suffers from a mild form of deafness, and that a motherly uncle is affected by type 2 diabetes. Diagnosis of type 1 diabetes was made and insulin treatment initiated. Antiislet cell autoantibodies (IAA, IA2, GAD) were negative and mitochondrial diabetes was excluded. The patient did not show any remission phase and metabolic control was mostly poor. Now, at 5.3 years of age, her average glycosylated hemoglobin is 8.7 % (normal values up to 6%). Her psychomotor development was normal until 2 yo, when she experienced atypical prolonged hypoglycemia with ataxia and hypotonia after an acute viral infection. The follow-up showed a retardation of psychomotor development (developmental age 2.7 years at age 5 years) and growth (height 3 SD, weight 1.6 SD). X-ray imaging identified signs of spondyloepiphyseal dysplasia in the whole skeleton. Mutation analysis of the EIF2AK3 gene was carried out via polymerase chain reaction amplification and direct sequencing of the gene. Based on the clinical data, a diagnosis of WRS was made. The patient carries a homozygous T to C exchange in exon 13 leading to the missense Ser 877 Pro mutation. Ser 877 is located in the catalytic domain and it is conserved among rat, mouse and human EIF2AK3 proteins. The mutation is not a polymorphism since it is absent in 50 normal controls (100 alleles). This is the third case of EIF2AK3 gene mutation related to WRS and confirms the crucial role of EIF2AK3 in pancreas islet and bone development in humans.

**AAAS mutations in patients with Allgrove Syndrome and sequence alterations of the same gene in a patient with iACTHR suggesting a new source or genetic heterogeneity for iACTHR.** C. Farmakidis, F. Sandrini, L.S. Kirschner, C.A. Stratakis. Unit on Genetics and Endocrinology, Developmental Endocrinology Branch, NICHD, Bethesda, MD.

Familial glucocorticoid deficiency due to corticotropin resistance consists of three distinct genetic syndromes, all inherited as autosomal recessive traits: mutations of the *MC2R* gene, isolated ACTH resistance (iACTHR) without *MC2R* mutations, and Allgrove or triple-A syndrome (AS). Our group collected families with iACTHR (N=5) and AS (N=7) to investigate the molecular basis of AS, and also to examine whether mutations of *AAAS* are associated with iACTHR. Sequencing analysis found no ACTHR receptor gene defects in any kindreds. Five of 7 AS families had *AAAS* mutations including a kindred with AS that was homozygous for a missense point mutation (43C>A, Gln15Lys) and showed a distinctly milder AS phenotype. While no mutations were found in the *AAAS* gene of iACTHR families, one iACTHR patient was found to be a heterozygote for a sequence change 503C>T in exon 5. cDNA analysis for this family showed an abnormal splice variant skipping exon 4 leading to a premature stop codon in the mother, also a carrier of the sequence change. In the case of the affected child's cDNA, while *B Actin* control primers yield PCR products, *AAAS* primers known to work do not yield full length cDNA amplicons for the gene, which suggests downregulated expression of the gene. Transfection experiments are on the way to distinguish if the exon 5 sequence change is a neutral sequence alteration or is instead a mutation that causes the post-transcriptional skipping of exon 4 and therefore contributes to the iACTHR phenotype. We conclude that *AAAS* was found mutated in families that we had previously mapped to 12q13. This gene was also found to have a coding sequence polymorphism/mutation in 1/5 of families with iACTHR indicating that there may be overlap in the genetic causes of the two syndromes, as well as genotype-phenotype correlation.

**Comparative analysis of 81 *NR0B1* mutations identified in adrenal hypoplasia congenita patients.** *J.K. Phelan, Y-H. Zhang, B-L. Huang, E.R.B. McCabe.* Pediatrics, UCLA School of Medicine and Mattel Children's Hospital at UCLA, Los Angeles, CA.

A wide variety of mutations in *NR0B1*, the gene encoding the nuclear receptor (NR) superfamily orphan member DAX1, are associated with X-linked adrenal hypoplasia congenita (AHC) and hypogonadotropic hypogonadism. In this study, we examined the types and distributions of all 71 published intragenic *NR0B1* mutations and an additional 10 novel mutations identified in our laboratory by sequencing amplified genomic DNA samples from patients with AHC. Of the 81 mutations, 42 (52%) were frameshifts and were distributed throughout the coding sequence. As many as 22 of these 42 frameshifts may have arisen by slipped mispairing errors of polymerases during DNA replication. Nonsense mutations, also distributed throughout the coding sequence, occurred in 22 of 81 mutations (27%). There are 18 codons in the genetic code, encoding 10 amino acids, which are susceptible to conversion into premature stop codons by single nucleotide substitutions. Among the nonsense susceptible codons in the *NR0B1* coding sequence mutations appeared to occur differentially: the most frequently mutated were TGG (tryptophan, 5/10 sites mutated) and TAC (tyrosine, 6/13 sites mutated). Missense alterations represented 15 of 81 mutations (18.5%). Of these, 14/15 occurred in the DAX1 carboxy terminal region similar to the ligand binding domain (LBD) of a typical NR. The exception to this distribution was the C200W mutation, which was found at the boundary of the DAX1 amino-terminus and the LBD. Of the 37 identified nonsense and frameshift mutations, none appear to have occurred by the deamination of methylcytosine at CpG dinucleotides. Lastly, two in-frame codon deletions were identified among the 81 mutations (2.5%), both in the LBD. In summary, protein truncating frameshift and nonsense mutations are distributed across the *NR0B1* coding sequence, while missense mutations and codon deletions are preferentially found in the LBD. Additionally, slipped mispairing errors during replication area a common cause of *NR0B1* mutations, while CpG methylcytosine deamination mutagenesis is rare.

**Gonadal Mosaicism in Pseudoachondroplasia: A Second Family with a COMP mutation of Asp518His. E.M.**

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Pseudoachondroplasia (PSACH, OMIM #177170) is a skeletal dysplasia caused by a mutation in the gene encoding cartilage oligomeric matrix protein (COMP). PSACH is characterized by short-limb dwarfism that becomes apparent in early childhood. More than 50 novel mutations have been identified in the conformationally sensitive type 3 calcium binding domains. While this condition is typically inherited in an autosomal dominant pattern, there are several reported cases of known or suspected somatic or gonadal mosaicism. At least three cases of gonadal mosaicism have been confirmed through mutational analysis. Two of these cases were due to mutations in exon 17B, the most common location of identified PSACH mutations, and the other was due to a mutation in exon 18A, G1577C, changing an aspartic acid to a histidine. We report a second unrelated family with the same mutation in exon 18A. Two sisters with typical findings of PSACH presented in early childhood and diagnosis was confirmed by mutational analysis using bi-directional sequencing (GeneDX, Inc., Rockville, MD). Neither parent in this family showed evidence of this mutation through restriction digest analysis in DNA obtained through peripheral blood. The possibility of germline mosaicism in one parent, while not confirmed, remains the most likely explanation for the recurrence of PSACH in this family. It is intriguing that the same mutation was found in two unrelated families, each with two affected daughters, due to gonadal mosaicism. Investigation of isolated or believed spontaneous cases of PSACH is warranted to assist in genetic risk assessment, as somatic or gonadal mosaicism has been identified in several cases.

**Analysis of CAG Repeats Expansion SCA7 in Patients with Usher Syndrome with Cerebellar Alterations. C.**

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The association of retinal degeneration, deafness and cerebellar alterations is present in several diseases, two of them are Usher Syndrome and Spinocerebellar Ataxia type 7 (SCA7). Types of cerebellar abnormalities seen in both diseases are very similar. Usher syndrome (USH) is an AR disorder characterized by congenital sensorineural deafness, vestibular dysfunction, and progressive retinitis pigmentosa. A chronic neurodegenerative process has been suggested in USH. Ataxia and cerebellar abnormalities have also been reported. In Colombia we carried out a neurological evaluation and MRI analysis in USH patients. We observed abnormal gait in 88,9% of USH1, in 66,7% of USH2 patients, abnormal coordination in 33,4% of USH1, in 58,3% of USH2. MRI analysis showed cerebellar abnormalities in 50% of USH1 and 75% of USH2 patients. SCA7 is a progressive neurodegenerative disorder caused by CAG expansion, characterized by cerebellar ataxia (100%) with retinal degeneration (83%), optic atrophy (25%), ophthalmoplegia (53%) and deafness (24%). Brain imaging showed marked atrophy in the cerebellum, particularly in the vermis. We studied 13 USH individuals with cerebellar abnormalities, representing 5 kindreds. Of the 13 individuals 8 (61.5%) were USH1 and 5 (38.5 %) USH2. The MRI findings were atrophy of the cerebellar vermis, enlargement of the cisterna magna, prominence of the folias and enlargement of the superior cerebellar cistern. These patients do not show genetic anticipation. We investigated the normal size range of the SCA7 gene by genotyping of normal Colombian individuals. The range of the number of CAG repeats was 11-17. We analyzed the CAG expansions of SCA7 in USH affected individuals, and did not find SCA7 mutation in any patient. Although we did not find any CAG expansion SCA7 in our USH patients, we consider that taking into account the clinical and neuroradiological findings seen in USH, it is important to emphasize the need to make a differential diagnosis with SCA7, especially in USH sporadic cases, considering the genetic heterogeneity observed in both diseases.

**X- and Y-bearing sperm are not different in CAG repeat size at Huntington's locus , confirming that gender-dependence of expansion arises postzygotically.** *I.V. Kovtun<sup>1</sup>, G. Welch<sup>5</sup>, H.D. Guthrie<sup>5</sup>, K.L. Anderl<sup>4</sup>, R.B. Jenkins<sup>4</sup>, C.T. McMurray<sup>1,2,3</sup>.* 1) Department of Molecular Pharm, Mayo Clinic, Rochester, MN; 2) Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN; 3) Molecular Neuroscience Program, Mayo Clinic, Rochester, MN; 4) Medicine and Pathology, Mayo Clinic, Rochester, MN; 5) Germplasm and Gamete Physiology Laboratory, USDA, Beltsville, MD.

Huntington's Disease (HD) is a progressive neurodegenerative disorder in which the underlying mutation is a CAG expansion encoding a polyglutamine tract. It has been documented that alterations in length of the CAG repeat after transmission are distinctly dependent on the gender of the transmitting parent. Large changes in repeat number are always associated with paternal transmission in HD. However, by following transmission in HD transgenic mice that model intergenerational instability, we found that CAG repeat size of the mutant human gene is different in male and female progeny from identical fathers. Males predominantly expand the repeat while females predominantly contract the repeat. This has raised the issue of whether the gender dependence of expansion in HD is "imprinted" in the germ cells of the parent or arises from the differences in the postzygotic stages. To distinguish between the two possibilities, we evaluated whether CAG repeat size differed in the X- and Y-bearing sperm of the founding father. Sperm from founding fathers were separated into distinct X- and Y-bearing populations by FACS analysis and the CAG repeat size in each population was evaluated. The purity of sorted cells was confirmed by FISH. We found no difference in CAG repeat distribution among the two sorted populations. We conclude that the gender dependence of CAG expansion in HD mice occurs postzygotically in the early embryo. Since the gender of the embryo is determined by the presence of X,X or X,Y chromosomes, our data support a model in which X- and Y-encoded factors contribute to the expansion.

**Trinucleotide and pentanucleotide repeats in 202 unrelated families with spinocerebellar ataxia.** *I. Silveira<sup>1</sup>, I. Alonso<sup>1,2</sup>, C. Miranda<sup>1,2</sup>, L. Guimarães<sup>1,2</sup>, M.C. Moreira<sup>1,2</sup>, P. Mendonça<sup>1</sup>, A. Ferro<sup>1</sup>, J. Pinto-Basto<sup>1</sup>, J. Coelho<sup>1</sup>, F. Ferreirinha<sup>1,2</sup>, L. Jardim<sup>4</sup>, P. Coutinho<sup>3</sup>, J. Sequeiros<sup>1,2</sup>.* 1) UniGENe, IBMC - Univ Porto, Porto, Portugal; 2) Lab. Genética Médica, ICBAS- Univ Porto, Portugal; 3) Serviço de Neurologia, Hospital São Sebastião, Feira, Portugal; 4) Hospital de Clínicas de Porto Alegre, Brazil.

Eleven neurodegenerative disorders characterized by spinocerebellar ataxia (SCA) are known to be caused by trinucleotide repeat (TNR) or pentanucleotide repeat expansions. We have (1) assessed trinucleotide and pentanucleotide sizes at all known loci, (2) determined frequency distributions of normal alleles and (3) of expansions, and (4) looked at genotype-phenotype correlations, in a large group of Portuguese and Brazilian patients with SCA. No differences were found in frequency distributions of normal alleles in both groups; DRPLA, MJD1, SCA1, SCA8, TBP, and SCA6 were the most polymorphic loci. Patients from 110 unrelated families with SCA showed TNR expansions at one of the loci studied; dominantly transmitted cases had (CAG)<sub>n</sub> expansions at the MJD1 (63%), SCA2 (3%), DRPLA (2%), SCA6 (1%) or SCA7 (1%) loci, or (CTG)<sub>n</sub> expansions at the SCA8 (2%) gene, whereas (GAA)<sub>n</sub> expansions in the FRDA gene were found in 64% of families with recessive ataxia. Isolated patients also presented TNR expansions at the MJD1 (6%), SCA8 (6%) or FRDA (8%) genes; in addition, an expanded allele at the TATA-binding protein (TBP) gene, with 43 CAGs, was present in a patient with ataxia and mental deterioration. Associations between frequencies of SCA2 and SCA6, and the frequency of large ANs were found in Portuguese and Brazilians, respectively. Interestingly, no association between the frequency of DRPLA and the frequency of large ANs was found in the Portuguese group. In conclusion, our results show that (1) a significant number of isolated cases with ataxia is due to TNR expansions, (2) expanded DRPLA alleles in the Portuguese may have evolved from an ancestral haplotype, and (3) small (CAG)<sub>n</sub> expansions at the TBP gene may be implicated in ataxia with mental deterioration.



**Dynamic of inclusion formation and processing of huntingtin.** *Y. Trottier<sup>1</sup>, A. Lunkes<sup>1</sup>, K.S. Lindenberg<sup>2</sup>, D. Devys<sup>1</sup>, G.B. Landwehrmeyer<sup>2</sup>, J.L. Mandel<sup>1</sup>.* 1) IGBMC/ULP/CNRS/INSERM U184, Strasbourg, France; 2) Department of Neurology, University of Ulm, 89075 Ulm, Germany.

Cellular and animal model systems for Huntingtons Disease (HD) have demonstrated that formation of nuclear inclusions (NIs) and cytoplasmic inclusions (CIs) depends primarily on the number of Glns and the length of the huntingtin (htt) protein expressed. In cellular models in particular, NIs were mainly observed with very truncated versions of htt and only in a few instances with full length htt in a low frequency of cells. We have previously reported that a tetracycline inducible cell model expressing truncated versions of htt or full length htt is able to produce both CIs and NIs at a high frequency (Lunkes et al. HMG 1998). Using our cell model, we show that CIs and NIs form with different dynamics thereby reflecting a different make-up of NIs versus CIs. On immunofluorescence analysis with antibodies that recognize different htt epitopes, we observe that in both, our cell model and in HD brains, NIs and CIs are heterogenous and are composed of different forms of htt cleavage products. Disaggregation of the NIs formed in the cell model reveals a major breakdown product that is highly ubiquitinated. We have identified a domain of 10 aminoacid in which htt cleavage most likely occurs and we are currently performing mutagenesis to assess whether inhibiting the cleavage will prevent NI formation.

**Slowly progressive cerebral dysfunction with selective striatal neuronal inclusions in a rat model transgenic for the Huntington disease mutation.** *O. Riess<sup>1</sup>, I. Schmitt<sup>2</sup>, H.P. Nguyen<sup>3,9</sup>, C. Holzmann<sup>1</sup>, T. Schmidt<sup>1</sup>, T. Walther<sup>4</sup>, M. Bader<sup>4</sup>, R. Pabst<sup>3</sup>, P. Kobbe<sup>3</sup>, D. Stiller<sup>6</sup>, A. Kask<sup>6</sup>, A. Vaarmann<sup>7</sup>, S. Rathke-Hartlieb<sup>8</sup>, J.B. Schulz<sup>8</sup>, U. Grasshoff<sup>1</sup>, I. Bauer<sup>1</sup>, M. Paul<sup>4</sup>, B. Landwehrmeyer<sup>5</sup>, X.J. Li<sup>9</sup>, St. von Hoersten<sup>3</sup>.* 1) Dept Med Gen, Children's Hosp, Univ Rostock, Rostock, Germany; 2) Dept Neurol, Univ Bonn, Bonn, Germany; 3) Funct and Appl Anatomy, Hannover Med School, Germany; 4) Max-Delbrueck-Center, Berlin, Germany; 5) Dept Neurol, Univ Ulm, Ulm, Germany; 6) Non-invasive Imaging, Leibnitz Inst of Neurobiol, Magdeburg, Germany; 7) Dept Pharmacol, Univ Tartu, Estonia; 8) Dept Neurol, Univ Tuebingen, Germany; 9) Dept Genetics, Emory Univ, School Med, Atlanta, USA.

Huntington's disease (HD) is a slowly progressive neurodegenerative disorder clinically characterized by involuntary movements, cognitive alterations and emotional and behavioural disturbances of mid-life onset. The disease is caused by a CAG trinucleotide repeat expansion in a gene of unknown function. To create an animal model for HD which is more suitable for behavioural and MR/PET-studies than mice we generated transgenic rats carrying a truncated HD-gene fragment including 51 CAG repeats under the control of the native HD promoter. These rats develop signs of HD including nuclear inclusions, neuropil aggregates, and altered tryptophan metabolism in the striatum and showed striatal shrinkage in MRI scans. Furthermore, they exhibit a slow progressive neurological phenotype composed of emotional disturbance, cognitive impairments, and motor dysfunction. This is the first transgenic rat model of a neurodegenerative disorder of the central nervous system closely resembling a human disease.

**Length of the CAG repeat in the SCA1 gene, but not Apo E status, modifies age of onset in spinocerebellar ataxia type 2 (SCA2).** *S.M. Pulst<sup>1</sup>, N. Santos<sup>2</sup>, L. Velazquez<sup>2</sup>, K.P. Figueroa<sup>1</sup>*. 1) Med, Div Neurology, Cedars-Sinai Medical Ctr, Los Angeles, CA; 2) CIRAH, Holguin, Cuba.

Expansion of a polyglutamine (polyQ) tract is a common mutation in several genetic late-onset neurological diseases. Although there is a clear inverse correlation between age of onset (AO) and repeat length, a significant variation is observed in the AO for a given CAG repeat length. For example, 37 repeats in the SCA2 gene are associated with an AO ranging from 15 to 65 years of age. To identify genetic modifiers of AO in SCA2 we analyzed age of onset and SCA2 repeat length in 300 patients from Holguin, Cuba. As reported for other polyglutamine diseases CAG repeat length contributed <60% of variation in AO in Cuban SCA2 patients. For each repeat class we determined mean age of onset and standard deviation (SD). Patients with an AO greater than the mean+1SD (n=31) constituted the 'delayed' onset group, patients with an AO of mean-1 SD (n= 33) the 'premature' onset group. We compared the frequency of alleles for the following candidate genes: SCA1, 3, 6, 7, apoE, hSKCA3, RAI1. Allelic association was detected only for CAG repeats in the SCA1 gene. Statistical significance was confirmed both for a threshold model for repeats > 30 (chi square test; (p=0.001) and for a model assuming a continuous effect of CAG alleles (Wilcoxon rank test; p=0.005). We could not confirm RAI1 as an age of onset modifier in this population. These results provide evidence for a possible association between longer alleles in the SCA1 gene and age of onset of SCA2. It is important to note that ataxin-1 and ataxin-2 have a very similar cellular distribution including strong expression in Purkinje cells and CA3 neurons of the hippocampus thus providing a biologic rationale for the observed association. Ataxin-1 proteins with long normal alleles may contribute to overall cellular stress over long time periods, thus aggravating the cellular dysfunction caused by pathologically expanded SCA2 repeats. Due to relative genetic homogeneity and similar environmental exposure the ataxia population in Holguin may represent a unique population to study age of onset modifiers for neurodegenerative diseases.

**Autosomal dominant susceptibility to tuberculosis in a multi-case pedigree.** *S.J. Campbell<sup>1</sup>, G.S. Cooke<sup>1</sup>, D.A. Lammas<sup>2</sup>, R. Barretto<sup>2</sup>, A.V.S. Hill<sup>1</sup>.* 1) WTC for Human Genetics, University of Oxford, UK; 2) Medical Research Council Centre for Immune Regulation, University of Birmingham, UK.

Clinical tuberculosis (TB) in people of British origin is now fairly uncommon in the UK and most often seen in people who have travelled from endemic regions of the world or who have an immunodeficiency, such as being HIV positive. Here we present a unique three-generation family from the UK who have no defined risk factors, yet have suffered from recurrent episodes of clinical TB. The episodes of TB seen in this family span 19 years, have occurred more than once in four members and in total have affected eight members of the family. One member has also been affected by a severe episode of *Aspergillus* infection. In the lungs, the alveolar macrophage is the first line of defence against both *Mycobacterium tuberculosis* and *Aspergillus*. The co-occurrence of both infections in this family is highly suggestive of a specific immune defect, possibly affecting some aspect of macrophage function.

Analysis of the pedigree suggests genetic susceptibility governed by an autosomal dominant, single major gene. Therefore, with the prior hypothesis that there is a dominant susceptibility locus in this family, a genome wide screen was carried out to locate it. 343 microsatellite markers spanning 22 autosomes were typed, the average distance between any two informative markers being 10cM (maximum 25cM). Multipoint analysis with GenehunterPlus, gave a maximum LOD score of 2.43, strongly supporting the presence of a dominant major susceptibility gene in this family.

**A nonsense mutation in CRYBB1 underlies autosomal dominant cataract linked to human chromosome 22q. D.S.**

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Non-syndromic hereditary cataract is a clinically and genetically heterogeneous lens disorder that most often presents as an autosomal dominant sight-threatening trait in childhood. Here we identify a locus for an autosomal dominant form of pulverulent cataract that maps to the b-crystallin gene cluster on chromosome 22q. Linkage analysis gave tentatively positive two-point LOD scores at markers D22S1167 ( $Z=2.09$  @  $q=0$ ) and D22S1154 ( $Z=1.39$  @  $q=0$ ). Haplotyping indicated that the cataract gene lay in the ~1.4cM interval between markers D22S1167 and D22S1154, which flank the genes for bA4-crystallin (CRYBA4) and bB1-crystallin (CRYBB1). Sequencing failed to detect any nucleotide changes in CRYBA4, however a G→T transversion was found in exon six of CRYBB1. Allele-specific PCR analysis showed that the G→T change co-segregated with affected individuals in the pedigree but was not present in twenty-five normal unrelated individuals. This single nucleotide change was predicted to introduce a chain termination mutation at codon 220 that changed glycine to a translation stop codon (G220X). Expression of recombinant human bB1-crystallin showed that the truncated G220X mutant was significantly less soluble in bacteria than its wild type counterpart. This study has identified the first mutation in CRYBB1 associated with autosomal dominant cataract in humans.

**Autosomal dominant canine malignant hyperthermia is caused by a mutation in the gene encoding the skeletal muscle calcium release channel (*RYR1*).** *M.C. Roberts<sup>1</sup>, J.R. Mickelson<sup>1</sup>, E.E. Patterson<sup>2</sup>, T.E. Nelson<sup>3</sup>, P.J. Armstrong<sup>2</sup>, D. Brunson<sup>4</sup>, K. Hogan<sup>4</sup>.* 1) Veterinary PathoBiology, Univ Minnesota, St Paul, MN; 2) Small Animal Clinical Sciences, Univ Minnesota, St. Paul, MN; 3) Anesthesiology, Wake Forest Univ, Winston-Salem, NC; 4) Anesthesiology, Univ Wisconsin, Madison, WI.

Malignant Hyperthermia (MH) is an inherited muscle disorder, characterized by rhabdomyolysis, generalized skeletal muscle contracture, cardiac dysrhythmia, renal failure, and typically an elevated body temperature, that develops when susceptible patients are exposed to succinylcholine or volatile anesthetics. All swine and 50% of human MH is caused by a mutation in the skeletal muscle calcium release channel of the sarcoplasmic reticulum, also designated as the ryanodine receptor (*RYR1*). To determine the molecular basis for canine MH, a breeding colony was established with a male Labrador Retriever who survived a reaction to halothane. He was mated to three unaffected females to produce four litters, and backcrossed to an affected daughter to produce one litter. An affected son of his was mated to an unaffected female to produce one litter. All dogs were phenotyped with an in vitro contracture test (IVCT), and they were diagnosed as MH susceptible (MHS) or MH normal (MHN). There were 21 MHS and 18 MHN pups in the five outcross litters. In the backcross litter there were two MHS and one MHN, and five pups that did not survive past two months. Pedigree analysis revealed MHS in this colony to be transmitted as an autosomal dominant trait. *RYR1* has been mapped to canine chromosome 1 (CFA01), and eight CFA01 microsatellite markers were tested for linkage to MHS. The marker closest to *RYR1*, FH2294, is linked to MHS at a distance of 5 cM with a LOD score of 8.9. The *RYR1* cDNA was partially sequenced and a polymorphism was found at base pair 1640 that changes amino acid 547 from a valine to an alanine. This polymorphism segregates with the affected individuals, and has a LOD score of 12.29 at  $Q = 0.0$  with the MHS locus. The polymorphism changes a highly conserved amino acid, which supports its role as the causative MH mutation.

**A novel gene for autosomal dominant Stargardt-like macular dystrophy with homology to the SUR4 protein family involved in very long chain fatty acid synthesis.** *R.L. Ritter<sup>1</sup>, L.A. Donoso<sup>2</sup>, A.O. Edwards<sup>1</sup>*. 1) Dept Ophthalmology, Univ Texas Southwestern, Dallas, TX; 2) Wills Eye Hospital, Philadelphia, PA.

**Purpose:** To describe a novel gene causing a Stargardt-like phenotype in a dominant macular dystrophy family and the exclusion of all known genes within the disease locus.

**Methods:** Meiotic breakpoint mapping in a family of 2,314 individuals enabled refinement of the location of the disease gene. The genomic organization and expression profile of known and putative genes within the critical region were determined using bioinformatics, cDNA cloning, and RT-PCR. The coding sequence of genes expressed within the retina was scanned for mutations using DNA sequencing.

**Results:** The disease-causing gene (*STGD3*) was further localized to 562 kb on chromosome 6 between D6S460 and a new polymorphic marker centromeric to D6S1707. Of the 4 genes identified within this region, all were expressed in the retina or retinal pigment epithelium. The only coding DNA sequence variant identified in these 4 genes was a 5 bp deletion in exon 6 of *ELOVL4*. The deletion is predicted to lead to a truncated protein with a net loss of 44 amino acids including a dilysine endoplasmic reticulum retention motif. The *ELOVL4* gene is the fifth known example of a predicted human protein with homology to mammalian and yeast enzymes involved in the membrane bound fatty acid chain elongation system. The genomic organization of *ELOVL4* and primer sets for exon amplification are presented.

**Conclusions:** *ELOVL4* causes macular dystrophy in this large family distributed throughout North America and implicates fatty acid biosynthesis in the pathogenesis of macular degeneration. The PRC-based assay for the 5 bp deletion will facilitate more accurate genetic counseling and identification of other branches of the family.

**North American Indian Childhood cirrhosis (NAIC or CIRH1A): fine mapping and sequencing of candidate genes.** *P. Chagnon, G.A. Mitchell, A. Richter.* Service de Génétique Médicale, Hôpital Sainte-Justine, Montréal, Québec, Canada.

NAIC/CIRH1A (MIM 604901) is an early onset, familial cholestasis, frequent in the Ojibway-Cree population of the Abitibi region in north-western Quebec, Canada. Our clinical collaborators identified a total of 30 cases. The disease typically presents with transient neonatal jaundice in a child who is otherwise well, and progresses to biliary cirrhosis requiring hepatic transplantation in childhood or young adulthood. Clinical and physiological investigations have failed to discover the underlying cause of NAIC. We mapped the NAIC locus by homozygosity to chromosome 16q22 using DNA samples from 13 patients. High-density mapping identified a five-marker haplotype (D16S3067, D16S752, D16S2624, D16S3025 and D16S3106) spanning 4.9 cM that is shared by all affected individuals (Btard et al. *Am. J Hum Genet* 67:222-228,2000). Recently we recruited a new patient, his mother as well as 2 siblings of a previously sampled patient to the study and obtained DNA from archival tissue samples from 6 patients. In order to better delimit the NAIC gene critical region, we have recently genotyped 6 other markers (D16S421, D16S3085, D16S3095, D16S3066, D16S3115 and D16S3086) mapped to 16q22. The observed recombination events indicate that the NAIC region is located between D16S3085 and D16S3095. Data in the Human Genome Browser (<http://genome.ucsc.edu/>) currently estimates the physical distance between these two markers at 2,3 Mb. This region contains around 60 genes. In order to identify the NAIC gene we are proceeding with a sequence based approach that compares gene sequence obtained in patient DNA with that of heterozygote parents and unaffected controls. The complete coding regions and some flanking intronic sequence of twelve genes, most preferentially expressed in liver (CA7, CES2, SLC9A5, HSD11B2, ATP6DV, PSMB10, LCAT, CDH1, HAS3, TAT, DHODH and PRP16) have now been sequenced. In addition, we also sequenced representative ESTs from 10 Unigene clusters that are expressed in fetal liver. Although the causal mutation of NAIC remains to be identified, we found many neutral SNPs that are associated with the disease and confirm the microsatellite markers analysis.



**A Novel mutation in TM4SF2 causes MRX58.** *F.E. Abidi<sup>1</sup>, E. Holinski-Feder<sup>2</sup>, G. Turner<sup>3</sup>, F. Kooy<sup>4</sup>, H.A. Lubs<sup>5</sup>, R.E. Stevenson<sup>1</sup>, C.E. Schwartz<sup>1</sup>.* 1) JC Self Res Inst, Greenwood Genetics Ctr, Greenwood, SC; 2) Medizinisch Genetisches Zentrum, Bayerstrasse 53, D-80335, Munchen, Germany; 3) University of Newcastle, Hunter Genetics, Waratah, New South Wales, Australia; 4) Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; 5) Department of Pediatrics/Genetic Division, University of Miami, School of Medicine, Miami, FL.

Approximately 210 XLMR conditions (136 MRXS and 74 MRX) have been mapped. To date, 34 genes have been cloned. Of these, 9 are MRX genes: FMR2, OPHN1, GDI1, PAK3, RSK2, IL1RAPL, ARHGEF6, TM4SF2 and MECP2. One of these genes, TM4SF2, maps to Xp11.4 and is a member of the transmembrane 4 superfamily. We have screened probands from 14 families (10 linked to Xp11.4 and 4 small families with no linkage data) for mutations in the TM4SF2 gene. We report a novel 2 bp deletion (564delGT) in MRX58, which segregates with MR in the family. This deletion causes a frame shift at amino acid 186 (FS186X) thereby resulting in stop codon six amino acids downstream. The deletion falls in the 2nd extracellular domain and results in a truncated protein missing the 4th transmembrane as well as the carboxy end of the gene. The phenotype observed in MRX58 consists of mild to moderate MR with no dysmorphic features and is not much different from the phenotype reported in a family with a truncating mutation removing a similar portion of the TM4SF2 (Zemni et al. 2000). This implies that loss of function of the TM4SF2 results in a phenotype comprised mainly of an impaired cognitive function. Furthermore, our finding also supports the hypothesis that many of the published XLMR conditions could be due to mutations in the same gene.

**In search of MFS2 gene, the second gene implicated in Marfan syndrome.** *C. Boileau<sup>1, 3</sup>, K. Dahan<sup>1</sup>, MC. Bouterin<sup>1</sup>, G. Jondeau<sup>2</sup>, O. Dubourg<sup>2</sup>, JP. Bourdarias<sup>2</sup>, C. Junien<sup>1, 3</sup>, G. Collod Beroud<sup>1, 3</sup>.* 1) INSERM U383, Hopital Necker Enfants Malades, Paris, France; 2) Service de cardiologie, Hopital Ambroise Pare, Boulogne, France; 3) Laboratoire central de Biochimie, d Hormonologie et de Genetique moleculaire, Hopital Ambroise Pare, Boulogne, France.

It is now well established that defects in the fibrillin gene located on chromosome 15 (FBN1) cause not only classic Marfan Syndrome (MFS), but also a large range of milder, overlapping phenotypes. MFS was the founding member of the "heritable disorders of connective tissue". Cardinal manifestations of this autosomal dominant syndrome involves the eye, skeleton, and cardiovascular systems. We have been investigating a large family of more than 200 subjects presenting with MFS. By excluding the FBN1 locus in this large French family we raised the issue of genetic heterogeneity in MFS and the implication of a second locus (MFS2). Linkage analyses, performed in this family with dispersed anonymous DNA markers, have localized MFS2 successively to a region of 9 cM then less than 7 cM between D3S1293 and D3S2335 which map at 3p24.2-p25. The analysis was enlarged to 20 new family members and 4 new dinucleotide and 4 tetranucleotide DNA markers were tested in the family. These data refined the genetic localization of MFS2 to an area of less than 1 cM between markers D3S1583 and D3S3700. This region has an estimated physical distance of less than 1,5 Mb. Combination of data from the different integrated maps shows less than 60 potential cDNA transcripts. We have constructed complete YAC and BAC contigs and the transcriptional map of the critical region and located 6 known genes that are not plausible candidates (RAR beta, THRB, RPL15, NR1D2, UbcH6, kappa-B-RAS) and 18 regional ESTs. Of these 18 ESTs, 7 represent unknown genes. We are currently searching for the complete sequence of corresponding genes by Race PCR.

**Homozygosity mapping in a Norwegian family of a new locus for autosomal recessive ataxia to 20q11-q13. L.**

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Autosomal recessive ataxia shows genetic heterogeneity with loci identified for 6p21-23, 8q13.1 (vit-E deficiency), 9q33.3 (Friedreich ataxia), 10q34, and 19p13.3.

A six-generation Norwegian inbred family with slowly progressive autosomal recessive ataxia (Kvistad PH, et al. *Acta Neurol Scand*, 1985; 71:295-302) has been re-investigated. Seven patients, aged 4-59, had delayed walking ability, and infantile ataxia with cerebellar atrophy on CT-scans, but otherwise normal electrophysiological examinations. Six patients, and 17 additional family members provided DNA for a genome-wide scan with 412 microsatellite markers at 10 cM spanning. Six additional markers from chromosome 20 were genotyped. Multipoint nonparametric (NPLall) analysis demonstrated significant linkage (p-value < 0.007) for markers in the 22 cM region between D20S112 and D20S119 (20q11-q13). Markers in the 4.7 cM region between D20S912 and D20S884 were homozygous in all affected individuals. The mapping of a new locus for ataxia extends previously reported genetic heterogeneity.

Acknowledgement:

We thank Jeff Trent at Cancer Genetics Branch, NHGRI, NIH, for providing expert laboratory facilities for this study. *Kvistad PH, et al Acta Neurol Scand., 1985; 71:295-302.*

**CHILD syndrome: molecular and functional analysis.** *K.-H. Grzeschik<sup>1</sup>, A. Koenig<sup>2</sup>, D. Bornholdt<sup>1</sup>, H. Engel<sup>1</sup>, R. Happle<sup>2</sup>.* 1) Dept Human Genetics, Philipps Univ, Marburg, Germany; 2) Dept Dermatology, Philipps Univ, Marburg, Germany.

CHILD syndrome (Congenital Hemidysplasia with Ichthyosiform Nevus and Limb Defects, MIM 308050, an X-linked dominant, male-lethal trait characterized by an inflammatory nevus that usually shows striking lateralization with strict midline demarcation as well as ipsilateral hypoplasia of the body is caused by mutations in the gene NSDHL (NAD(P)H steroid dehydrogenase-like protein) encoding an enzyme functioning in the distal Kandutsch-Russell pathway for cholesterol biosynthesis. NSDHL maps to Xq28. Heterozygous nonsense or missense mutations, scattered over the coding region, were identified in 19 patients with CHILD syndrome. The severe developmental defects in CHILD syndrome may originate from teratogenic by-products of the cholesterol biosynthetic pathway or a disturbance of the synthesis of steroid hormones. The pattern of lateralization observed in most CHILD syndrome patients appears to be unique and difficult to explain. In fibroblasts from the affected side of different patients, X-chromosome inactivation is skewed in different proportions, however, on the non-affected side only the X with the intact NSDHL appears to be active. This pattern might reflect a defect of the mixing of differentially inactivated cells during gastrulation or, alternatively, asymmetric gene expression in and around the node which is responsible for left-right determination in vertebrates might result in lateralized selection against NSDHL-deficiency in a population of cells with X-chromosomes inactivated at random. Two murine X-linked dominant male-lethal traits, bare patches (Bpa) and striated (Str) had previously been associated with mutations in *Nsdhl*. The skin defect in these mice provides a model for the development of ichthyosis. However, the lack of lateralization of the skin defect in Bpa and Str questions the role of the mouse as a model for the study of left/right determination in early human development. CHILD syndrome heralds a gene that plays a pivotal role at an early stage of human development, when the formation of the primitive streak confers a bilateral symmetry on the embryonic disk.

**A defect in a novel Nek-family kinase causes cystic disease in the mouse and in zebrafish.** S. Liu<sup>1</sup>, W. Lu<sup>1</sup>, S. Kuida<sup>1</sup>, T. Obara-Ishihara<sup>2</sup>, I. Drummond<sup>2</sup>, D.R. Beier<sup>1</sup>. 1) Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; 2) Renal Division, Massachusetts General Hospital, Boston, MA, United States.

The murine autosomal recessive juvenile cystic kidney (*jck*) mutation results in progressive polycystic kidney disease (PKD). Using a positional cloning strategy, we identified a mutation in a novel gene that is a member of the the Nek (NIMA-related-kinase) protein family, which we call *Nek8*. The mutation results in a glycine-to-valine substitution in a highly conserved motif contained in the presumptive regulatory domain of the protein. To prove that a defect in the *Nek8* gene causes cystic disease, we performed a cross-species analysis taking advantage of the evidence that morpholino anti-sense oligos can be used to abrogate gene function in developing zebrafish. Treatment of embryos with an oligo corresponding to the 5' end of the zebrafish ortholog of *Nek8* resulted in the formation of pronephric cysts.

Immunohistochemical analysis of an HA-tagged *Nek8* construct transfected into COS cells demonstrates that the protein is localized in the cytoplasm. Transfection of either the *jck* mutant *Nek8* gene or a construct mutated in the kinase domain results in markedly enlarged and multinucleated cells. These enlarged cells demonstrate a striking loss of actin stress fibers. Mutant *jck* kidneys do not show multinucleation in vivo and immunohistochemical analysis does not show mislocalization of membrane proteins. However, EM analysis reveals that, prior to cyst formation, there is a dramatic expansion and disorganization of the basal labyrinth and the collecting duct epithelia appear to be lifting off the basement membrane. We suggest that a disruption in actin cytoskeletal assembly by the *jck* mutation affects cellular transport functions, which results in the development of cysts. Further, we suggest that a comparative analysis of gene function in different model systems represents a powerful means to annotate gene function.

**Characterisation of a novel gene on distal 11q as a potential candidate for Hypoplastic Left Heart Syndrome.**

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Hypoplastic Left Heart Syndrome (HLH) is a severe congenital heart defect, which affects approximately 1 in 5,000 live-born. It is characterised by underdevelopment of the left ventricle with severe atresia of the aortic valve and / or mitral valve. At present the cause of HLH is unknown but there is evidence for a genetic aetiology in a proportion of cases. HLH has been associated with 11q23-qter deletions, (Jacobsen Syndrome), which is characterised by cardiac defects (including HLH), trigonocephaly and thrombocytopenia. We have narrowed down the critical region for cardiac defects within 11q by investigating three patients with HLH, who had terminal deletions of 11q. Using FISH and microsatellite analysis, the breakpoints were mapped and defined an 8Mb region of haploinsufficiency on distal 11q. Within this critical region, genes and ESTs were assessed and one was chosen for further characterisation. Using RACE, full-length cDNA sequence was obtained. Sequence analysis showed that it was a member of a cell adhesion molecule gene family that was highly conserved between species. Northern blot analysis showed that the highest expression was in brain, skeletal muscle, heart, lung, kidney and placenta. More detailed expression analysis was performed using tissue in situ hybridisation on human embryonic tissue sections between Carnegie stages 13-21 (28-52 days post conception). Expression was highest in the neural tube, skeletal muscle and the aortopulmonary valves and atrioventricular cushions of the heart. We are currently investigating isolated and familial cases of HLH for mutations and deletions of this gene. Conclusion: we have significantly refined the critical region on chromosome 11q associated with HLH and identified a gene, the expression of which strongly implicates it as a candidate for this phenotype.

Program Nr: 2569 from the 2001 ASHG Annual Meeting

**Profound effects on neural development obtained by modulating ATRX dosage.** *D.J. Picketts, N.G. Bérubé, M. Jagla, C.A. Smeenk, Y. DeRepentigny, R. Kothary.* Molecular Medicine Program, Ottawa Health Research Inst, Ottawa, Ontario, Canada.

ATRX, a SWI/SNF-like protein, causes severe X-linked mental retardation (XLMR) and a wide range of developmental defects when mutated. Evidence from patient mutations indicate that that the major phenotypic features are sensitive to ATRX dosage. To examine the molecular basis of this dosage sensitivity we have overexpressed ATRX in transgenic mice and assessed its role in neural development. These mice display a variable phenotype including early embryonic lethality and malformation of the neuroepithelium that is suggestive of a defect in cell proliferation. This study demonstrates that ATRX dosage is crucial for cortical development and provides a basis for the XLMR observed in patients.

Program Nr: 2570 from the 2001 ASHG Annual Meeting

**A novel gene is disrupted by a Cornelia de Lange-associated translocation breakpoint at 3q26.3.** *E. Tonkin, M. Smith, P. Eichhorn, D.M. Hagan, S. Herrell, M. Lusher, M. Ireland, J. Burn, T. Strachan.* Institute of Human Genetics, University of Newcastle, Newcastle, UK.

Cornelia de Lange Syndrome (CDLS) is a developmental malformation syndrome characterised by mental handicap, growth retardation, distinctive facial features and limb reduction defects. Although most cases occur sporadically, estimated sib recurrence risks and transmission patterns in rare multicase pedigrees have suggested dominant inheritance. A CDLS locus, CDL1, has been postulated at 3q26.3-q27 based on phenotypic overlap with the duplication 3q syndrome whose critical region has been reported to be 3q26.2-q27, and by the identification of a classical CDLS patient with a balanced de novo t(3;17)(q26.3;q23.1) translocation. We have constructed YAC and BAC/PAC-based contigs for the CDL1 region of chromosome 3, defining the breakpoint. We have mapped a variety of genes in the breakpoint region, including a novel gene with a large transcription unit which is disrupted by the translocation. Northern hybridisation has revealed multiple hybridisation bands with prominent bands in kidney and placenta. The gene has multiple exons spanning more than 1 Mb and is predicted to produce multiple isoforms through alternative splicing/polyadenylation. On-going studies of the expression pattern during early development and mutation-screening will be reported.



**Three novel  $\beta$  thalassemia mutations and other prevalent mutations found in the Celtic (Irish and Scottish) populations.** *P.A. Bignell<sup>1</sup>, A. Jani<sup>2</sup>, C.A. Fisher<sup>1</sup>, S.M. Jobanputra<sup>3</sup>, M. van Mourik<sup>3</sup>, E.A. Chalmers<sup>3</sup>, N.P. Lucie<sup>4</sup>, J.M. Old<sup>1</sup>.* 1) Haemoglobinopathy Laboratory, Weatherall Institute of Molecular Medicine, Oxford, Oxon, UK; 2) Kennedy Galton Institute, Northwich Park, London, UK; 3) Dept of Haematology, Royal Hospital for Sick Children, Glasgow, UK; 4) Dept of Haematology, Western Infirmary, Glasgow, UK.

Thalassemia in Britain was thought to be present predominantly in the ethnic groups that have migrated into the country. However, both  $\alpha$  and  $\beta$  thalassemia has been found in the indigenous population. We have investigated the molecular basis of  $\beta$  thalassemia found in the Celtic population. Fourteen different families with no known foreign ancestry and presenting with typical  $\beta$  thalassemia trait were characterised. DNA was analysed for  $\beta$  thalassemia mutations using a combination of allele specific priming and direct sequencing of genomic DNA amplified by the polymerase chain reaction. Three mutations previously described on mainland Europe were identified. Two were Mediterranean mutations: codon 39 (C $\rightarrow$ T) was found in 9 families and IVS1-110 (G $\rightarrow$ A) in a single family. The third mutation, IVSII-850 (G $\rightarrow$ A), was also found in a single family and has been previously reported in a family of North European origin. Three novel mutations were identified. The mutations codon 131 (+A) and codon 131 (C $\rightarrow$ T) were found in two Scottish families and the mutation codon 125/126 (+A) was found in an Irish family. The prevalence of the two Mediterranean mutations could have resulted from population migration at the time of the Roman occupation of England, but as with the novel mutations, an independent origin of these mutations in the Scottish and Irish populations is possible. The  $\beta$  globin haplotypes associated with these mutations are being studied to investigate this further.

**SAETHRE-CHOTZEN SYNDROME: report on 4 Brazilian families without mutation on TWIST gene. S.R.D.**

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Saethre-Chotzen syndrome (acrocephalosyndactyly type III) is an autosomal dominant craniosynostosis syndrome, with high penetrance and variability of expression. This syndrome is characterized by both premature fusion of the calvarial bones (craniosynostosis), facial asymmetry, hypertelorism, blepharoptosis, deviated nasal septum, low frontal hairline, small ear with prominent crura, brachydactyly, broad great toes, and partial cutaneous syndactyly. Mutations in Twist gene ( 7p21 ) have been reported in some patients affected by SCS. Four families with ACS III were screened for Twist mutations. The analysis was carried out by direct DNA sequencing of PCR amplified products for exon 1 in the proband of each family. No Twist mutations were found. This result suggested that this four SCS families may have mutations in other genes of the same developmental pathway. The FGFR2 and FGFR3 genes appears to be implicated in interactions with Twist. Additional screening of these sequences may identify other critical regions involved in the pathogenesis of Saethre-Chotzen syndrome in these families.

**Mutational analysis of DM domain genes in the region on 9p associated with 46,XY gonadal dysgenesis. C.**

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Mutations in the coding region of the mammalian master sex-determining gene, SRY, account for less than 20% of cases of 46,XY complete gonadal dysgenesis. A similar condition is associated with deletions of the distal short arm of chromosome 9, suggesting that mutations in a locus mapping to band 9p24.3 may be responsible for isolated male-to-female sex reversal in humans. The gene DMRT1, located immediately proximal to the centromeric boundary of the minimal deleted region (at less than 700 kb from the 9p telomere), appears as an interesting candidate because it contains a unique domain, termed DM, that is conserved in two genes involved in invertebrate sex differentiation. Mouse *Dmrt1* is required for testis differentiation. However, anomalies occur postnatally in the mouse knock-out model and previous extensive screens detected only a single potential mutation in a sex-reversed patient. This suggested that the human 9p-deletion phenotype may be a contiguous gene syndrome, possibly involving other DM-containing genes in the region. We sequenced the DMRT1 DM domain in 104 sex reversed patients and found several single nucleotide variants, including a potential de novo missense mutation in a female with 46,XY complete gonadal dysgenesis and in her cryptorchid brother. We also characterised and screened an additional DM domain gene, DMRT3, located 8 kb downstream of DMRT1, and an unrelated, anonymous gene located upstream of it. No mutations were detected in these genes. Although not conclusive, these results strengthen the hypothesis that DMRT1 may be directly involved in human sexual development.

**A novel insulin-like 3 (*INSL3*) gene mutation associated with human cryptorchidism.** P. Marin, A. Ferlin, A. Rossi, E. Moro, L. Bartoloni, C. Foresta. Dpt of Medical and Surgical Sciences, University of Padova, Italy.

Cryptorchidism (impaired testicular descent) occurs in 1-2% of full-term male newborns and it is associated with a high risk of infertility and testicular cancer. The outgrowth of gubernaculum and regression of the cranial suspensory ligament result in the descent of the testes into the scrotum. The aetiology of this disorder is for the most part unknown and genetically heterogeneous. Male mice *Ins13*<sup>-/-</sup> exhibit cryptorchidism due to alteration of gubernaculum development, suggesting *INSL3* as putative gene for such disease. The *INSL3* gene consists of two exons coding for a proprotein characterised by a signal peptide, a B chain, a C-peptide and an A chain. The signal and the C peptide are cleaved during the hormone maturation. We found two aminoacid substitutions in heterozygosis in a patient with cryptorchidism and infertility, the common polymorphism A60T in exon 1 and a novel C to T transition in exon 2, which changes Arg 102 in Cys (R102C). This change was found in one allele over 1146 in a population of ex-cryptorchid patients and controls. The mutated arginine belongs to a conserved stretch of positively charged aminoacids near the C peptide cleavage site. The change of the aminoacid sequence might abolish the processing of C peptide. The *INSL3* receptor links to the A chain N-ter, and, if the C peptide is not excised from the A chain, the hormone will not be able to properly stimulate its receptor. A and B chains are linked by two disulfide bonds, and an extra disulfide bond is present within two A chain cysteines. The presence of an additional Cys in proximity of the N-ter of A chain might interfere with the forming bonds. If the Cys in the mutated residue forms a disulfide bond with one of the other Cys there will be a lacking bond between the chains A and B or an altered A chain conformation. Moreover, even if the cleavage site between C peptide and the A chain is recognised, the cleaved C peptide, attached to the A chain because of the disulfide bond, will sterically disturb the link to the receptor. The low frequency of this base change and the physiochemical differences between R and C suggest that this is a deleterious mutation.

**A Familial Mutation in the Testis-Determining Gene SRY Shared by an XY Female and her Normal Father. B.K.**

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In humans, mutations in the testis-determining gene SRY result in XY sex reversal with pure gonadal dysgenesis (PGD). However, only about 25% of the cases of PGD can be explained by mutations within the SRY open reading frame, suggesting the existence of other sex-determining genes. Although SRY is known to bind and bend DNA, its target and mode of action remain elusive. Here we describe a novel mutation in SRY in codon 127, resulting in a Y to F substitution in the protein. This sequence variant was found not only in the XY female patient, but also in her father who is a phenotypically normal male. The substitution affects a highly conserved Tyr residue in the HMG box of SRY, in which a *de novo* mutation has been previously described which caused XY sex reversal with PGD. The fact that another missense mutation in this codon resulted in sex reversal suggests that our observed variant is not a polymorphism. The allelic variant of SRY transmitted in this family and shared by both a phenotypic female (proband) and a phenotypic male (proband's father) emphasizes the effects of modifier genes. In particular, it illustrates the importance of the interaction of specific alleles of polymorphic sex-determining genes on sexual development.

**The Epstein syndrome: a further renal disorder due to mutations in the nonmuscle myosin heavy chain 9 gene.**

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Epstein syndrome (EPTS) is an autosomal dominant disease characterized by nephritis, mild hearing loss, and thrombocytopenia with giant platelets. Renal and hearing abnormalities were indistinguishable from those seen in Fechtner syndrome (FTNS), an Alport-like variant. Macrothrombocytopenia is similar to that described not only in FTNS but also in May-Hegglin anomaly (MHA) and Sebastian syndrome (SBS), three disorders due to mutations in the nonmuscle heavy chain myosin IIA (MYH9). The clinical features of EPTS and the chromosomal localization of the respective gene in the same region as MYH9 suggested that this disorder could be allelic with the other giant platelet disorders. We identified MYH9 missense mutations in two familial cases of EPTS. In both families, an Arg702His substitution was found. An amino acid substitution at the same codon (Arg702Cys) has been previously identified in a FTNS patient. Notably, while the mutated thiol reactive group of Arg702Cys may lead to intermolecular disulfide bridges, with consequent formation of the inclusions typical of FTNS, the Arg702His mutation does not allow the protein to aggregate and give rise to Dohle-like bodies, which are indeed absent in EPTS. Finally, in a patient previously diagnosed as EPTS, we identified a de novo Asp1424His substitution identical to that reported in a FTNS patient. An accurate cytological reevaluation allows us to classify this patient. Thus, our results extend the allelic heterogeneity of MYH9 mutations and contribute to clarify the pathogenesis of several clinically distinct syndromes.

**Mutational analysis of the coding and non-coding regions of MECP2 gene, parental origin and X-inactivation studies, in a large cohort of Italian RTT subjects.** *L. Giunti, S. Guarducci, U. Ricci, I. Sani, M.L. Giovannucci Uzielli.* Genetics and Molecular Medicine University of Florence Department of Paediatrics (Italy).

Two years after the identification of the first mutations of MECP2 gene in RTT patients (Amir et al., 1999), many points remain unclear regarding the pathogenic mechanism of MECP2 gene and the apparent genetic heterogeneity of this disorder. Direct Mesp2 sequencing analysis reveals missense, nonsense and frameshift mutations only in a part of RTT subjects, both with classic and non-classic phenotypes. We have already screened a large cohort of RTT subjects, and found that 78% of them presented mutations (82/105). We had only one familial event (normal mother and affected daughter) and 80 isolated, unrelated, RTT affected girls. We have now in progress a deletion analysis of RTT subjects without apparent MECP2 mutation of the coding and non-coding regions, by using Fluorescence in Situ Hybridization. Parental origin of isolated MECP2 mutations by intronic polymorphisms analysis, and X-inactivation studies complete our scientific involvement in this disorder.

**Gene conversion events in adult-onset Spinal Muscular Atrophy.** *R. Mazzei<sup>1</sup>, F.L. Conforti<sup>1</sup>, A. Gambardella<sup>1,2</sup>, A. Magariello<sup>1</sup>, A. Patitucci<sup>1</sup>, A.L. Gabriele<sup>1</sup>, D. Pirritano<sup>2</sup>, A. Labate<sup>2</sup>, M. Muglia<sup>1</sup>, P. Valentino<sup>2</sup>, F. Bono<sup>2</sup>, M. Zappia<sup>2</sup>, A. Quattrone<sup>1,2</sup>.* 1) Institute of Experimental Medicine and Biotechnology National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, School of Medicine of Catanzaro, Italy.

**Objective:** The survival motor neuron (SMN) gene is involved in the pathogenesis of spinal muscular atrophy (SMA). We present the molecular analysis of three patients with adult onset SMA, who carried an apparent homozygous deletion of telomeric SMN (SMNt) exon 7 but not of exon 8. **Materials and Methods:** Patient 1 is a sporadic case, patients 2 and 3 are two brothers. SMN exon 7 and exon 8 were amplified and subsequently digested with DraI for exon 7 and DdeI for exon 8. We found homozygous deletion of the SMNt exon 7 but not of the exon 8 in our SMA patients. In order to amplify a 1,010 bp fragment, we used the primers R111 and 541C1120. The PCR products were further amplified with primers for exon 8, and the fragments obtained were digested with DdeI. **Results:** In all patients the SMNc exons 7 and 8 were present. To distinguish between deletion and a sequence conversion event of telomeric exon 7, we performed an amplification of the region surrounding exon 7 and 8. The resulting 1,010 bp fragments were subjected to nested PCR of exon 8. The subsequent analysis by DdeI digestion showed products for both telomeric and centromeric copies of exon 8. These findings indicate a gene conversion event as the site for primer R111 was retained at least in one of two alleles. **Discussion:** The results of our study have demonstrated that SMA patients with a milder type IV phenotype and an isolated deletion of exon 7 as detected by traditional methods carry a gene conversion event when deeply investigated. As far as we are aware, this is the first evidence of a conversion event in adult-onset SMA in which exon 7 of SMNt is converted to SMNc. **Conclusions:** Our results provide the first evidence that a conversion event may be also associated with adult-onset SMA phenotype, and further support the notion that a gene conversion event may be correlated with a milder SMA phenotype and a later onset of disease.



**A novel mutation in the NOTCH3 gene in an Italian family with CADASIL.** *A.M.F. Patitucci<sup>1</sup>, R. Mazzei<sup>1</sup>, F.L. Conforti<sup>1</sup>, A. Magariello<sup>1</sup>, N. De Stefano<sup>3</sup>, A.L. Gabriele<sup>1</sup>, A. Labate<sup>2</sup>, M. Zappia<sup>2</sup>, A. Gambardella<sup>1,2</sup>, M. Muglia<sup>1</sup>, A. Federico<sup>3</sup>, A. Quattrone<sup>1,2</sup>.* 1) Institute of Experimental Medicine and Biotechnology, National Research Council, Mangone, Cosenza, Italy; 2) Institute of Neurology, Department of Medical Sciences, University of Catanzaro, Catanzaro, Italy; 3) Department of Neurological Sciences, University of Siena, Siena, Italy.

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an hereditary syndrome with adulthood onset, characterized by recurrent strokes. Mutations within the Notch3 gene have been identified as the underlying genetic defect associated with CADASIL. We identified two patients belonging to a large pedigree in whom clinical and laboratory findings were suggestive of CADASIL. We undertook mutation analysis of the Notch3 gene in the two patients. Furthermore, patients underwent to magnetic resonance spectroscopic imaging (MRSI). Sequence analysis of exon 6 showed a new missense mutation CGC>TGC in codon 332, resulting in the replacement of an arginine residue with a cysteine. The restriction enzyme MvnI recognizes the sequence CGCG, and the C>T transition causes the loss of an MvnI restriction site in the mutant PCR product. MvnI cleaves the wild type PCR product of 279 bp into 224 bp and 55 bp digestion fragments, but does not cleave the corresponding region of the Notch3 gene in CADASIL patients. We demonstrated the occurrence of this mutation in the affected subjects of the family, in none of the unaffected members of the family, and in none of the 200 control chromosomes examined. Here we describe a novel mutation occurring in exon 6, a location not previously recognised in CADASIL patients. Similar to previously reported mutational modifications in other exons of Notch3, this mutation is located at the 5' end of the gene and is characterised by the gain of a cysteine residue. It has been proposed that these modifications to the primary sequence affect the folding of Notch3 or induce inappropriate disulfide bonding between Notch3 and other cysteine-containing proteins.

**Isolation, characterisation and mutation analysis of a novel gene in the Noonan Syndrome critical region.** *K. Kalidas<sup>1</sup>, A.H. Crosby<sup>1</sup>, A.M. Ion<sup>1</sup>, H. Kremer<sup>2</sup>, I. Van der Burg<sup>2</sup>, H.G. Brunner<sup>2</sup>, K. Montgomery<sup>3</sup>, R. Goldberg<sup>4</sup>, R.S. Kucherlapati<sup>4</sup>, M.A. Patton<sup>1</sup>, S. Jeffery<sup>1</sup>. 1) Medical Genetics Unit, St George's Hospital Medical School, London, UK; 2) Department of Human Genetics, University Hospital Nijmegen, Nijmegen, Netherlands; 3) Molecular Genetics Department, Albert Einstein College of Medicine, Bronx, New York, USA; 4) Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.*

Noonan syndrome is an autosomal dominant condition characterised by cranio-facial dysmorphism, congenital heart defects and short stature. We have previously mapped a gene for NS to chromosome 12q24.1. We have used RT-PCR, RACE and cDNA library screening to identify candidate genes for NS that map to this region. Here we describe the structure, chromosomal location and expression pattern of a novel gene. We extended the sequence of an EST (Accession ID:10948129) with 5' and 3' RACE. A cDNA library was screened with a probe generated from this sequence and a clone was identified. The 1.6 kb clone was sequenced and comparison to genomic sequence showed that the gene lacked introns and contained a 300bp ORF. The gene was mapped to PAC P784A12 and BACs B385N17 and B435A10. To determine the expression pattern of this gene a 1.2kb sequence was hybridised against a multiple human tissue mRNA blot. The gene is expressed at high levels in heart, lung and placenta. SSCP was used to evaluate this gene for disease specific mutations in a cohort of 20 NS patients. No variants were identified, confirming that this gene does not contribute to the development of Noonan syndrome.

**Mutation analysis of the MECP2 gene in Southern Italy.** *F.L. Conforti<sup>1</sup>, R. Mazzei<sup>1</sup>, A. Fiumara<sup>2</sup>, A. Magariello<sup>1</sup>, A. Patitucci<sup>1</sup>, A.L. Gabriele<sup>1</sup>, G. Barone<sup>2</sup>, R. Nistico<sup>3</sup>, L. Mangone<sup>3</sup>, M. Muglia<sup>1</sup>, L. Pavone<sup>2</sup>.* 1) Institute of Experimental Medicine and Biotechnology, National Research Council, Mangone, Cosenza, Italy; 2) Department of Paediatrics, University of Catania, Catania, Italy; 3) Institute of Neurology, Department of Medical Sciences, University of Catanzaro, Catanzaro, Italy.

Rett syndrome is a childhood neurodevelopmental disorder almost exclusively affecting females. A gene for Rett syndrome (MECP2) has been identified on the X chromosome in q28 that binds to single methylated CpG base pairs throughout the genome and silences other genes. MECP2 encodes the methyl-CpG-binding protein 2, an ubiquitous DNA-binding protein that is thought to act as a global transcriptional repressor. This gene consists of 4 exons encoding 486 aa and shows a highly conserved large 3'UTR region that has been suggested to have regulatory functions. We report a detailed analysis by direct sequencing, of the MECP2 gene in 39 patients from southern Italy with classical or atypical Rett syndrome. Mutations in MECP2 were found in 11 of 15 classical Rett patients (73.3%), in 2 of 6 (33.3%) cases with variant form, and in 1 of 18 Rett-like patients (5.6%). Two novel mutations, not found in 100 control chromosomes, were detected. The first mutation, P376S (1126 C>T), was identified in a patient with classical Rett syndrome and the second, S388P (1162 C>T), was found in a patient with atypical Rett phenotype. To determine the role of X Chromosome Inactivation (XCI) contribute in phenotypic variability in Rett patients, we also evaluated XCI in patients with MECP2 mutations using the AR methylation assay. Our data indicate that the random XCI is associated with most classical Rett patients. In conclusion, our study shows a heterogeneous spectrum of disease-causing MECP2 mutations in a high proportion (73.3%) of classical Rett patients, confirming MECP2 is the major locus involved in the pathogenesis of Rett syndrome.

**Gene mutation and expression in Ellis van Creveld Syndrome.** *M. Galdzicka<sup>1</sup>, J.A. Egeland<sup>2</sup>, M.G. Hirshman<sup>1,3</sup>, S. Patnala<sup>1</sup>, B.M. Martin<sup>3</sup>, W.J. Kleijer<sup>4</sup>, E.I. Ginns<sup>1</sup>.* 1) Brudnick Neuropsychiatric Research Institute, Department of Psychiatry, University of Massachusetts Medical School, Worcester, MA; 2) Amish Study, Department of Psychiatry, University of Miami School of Medicine, Miami, FL; 3) Clinical Neuroscience Branch, NIMH, NIH, Bethesda, MD; 4) Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Ellis van Creveld syndrome (EvC) is an autosomal, recessively inherited skeletal disorder manifesting with dwarfism, polydactyly, ectodermal dysplasia, congenital heart disease, and occasionally renal failure. The gene for EvC syndrome, located on human chromosome 4p16.1, has been identified. We have investigated the differential expression of EVC gene in patients from the Netherlands and Old Order Amish from Pennsylvania. We found that some patients having the mutation in the splice acceptor site of intron 13 have either a) two transcripts, one having exon 13 extended by 115 bp and the other with exon 13 spliced out, or b) only one transcript having exon 13 extended by 115 bp. One of the Amish patients who does not have the mutation in the splicing site of exon 13 yields transcripts with additional exons that introduce stop codons in the middle of the protein or transcripts with no alternative splicing. Abnormal splicing of pre-mRNA observed in this Amish individual might be a mechanism responsible for the disease in the Dutch patients. We also examined the genomic DNA sequence for the occurrence of tissue specific transcripts and we identified a mutation that may be responsible for the tissue specific symptoms. However, the expected tissue-specific transcripts have not been observed in fibroblasts or lymphoblasts. Our results suggest that (1) alternative splicing of the EVC gene is tissue specific, and (2) that other factors such as differential expression and/or heterogeneity of alternative splicing may be responsible for a variety of clinical manifestations observed in EvC syndrome.

**Genotype phenotype correlations suggest a contiguous gene syndrome in retinoblastoma patients with germline 13q14 deletion.** *O. Baud*<sup>1</sup>, *F. Doz*<sup>2</sup>, *V. Cormier-Daire*<sup>1</sup>, *C. Turleau*<sup>1</sup>, *B. Bardoni*<sup>3</sup>, *A. Munnich*<sup>1</sup>, *S. Lyonnet*<sup>1</sup>, *A. Tullio-Pelet*<sup>1</sup>. 1) Department of Genetics, Necker Enfants Malades Hospital and INSERM U-393, Paris, FRANCE; 2) the Department of Paediatric Oncology, Institut Pierre et Marie Curie, Paris, FRANCE; 3) IGBMC INSERM U-184 Strasbourg, FRANCE.

Retinoblastoma is the most common malignant intra-ocular tumour of children in developed countries (1/20 000 live births). The retinoblastoma susceptibility gene RB1 located in band 13q14.2 encodes a nuclear phosphoprotein. While most RB1 gene mutations are point mutations, some retinoblastoma cases result from a cytogenetically detectable interstitial deletion encompassing the RB1 locus. The phenotype of such patients varies widely from isolated retinoblastoma to severe neurological impairment, occasionally associated with intra-uterine growth retardation and dysmorphic features suggesting the involvement of contiguous genes. We have studied the breakpoints and genotype/phenotype correlations of 13 patients with constitutional and cytogenetically detectable rearrangements of band 13q14. Chromosomal rearrangements included 11 de novo 13q14 interstitial deletions, and 2 de novo translocations involving chromosome 13q. Among deletions, 9/11 (82%) and 2/11 (18%) were of paternal and maternal origin respectively. In the four deleted patients with normal psychomotor outcome, the deletion spanned the D13S291 to D13S1268 interval (10cM). In contrast, patients with mental retardation presented deletions extending centromeric from locus D13S291 to locus D12S1297. Interestingly, the critical interval between these two markers (2.7 cM), includes the NUFIP1 locus, encoding a protein interacting with FMR1, which could be therefore regarded as a candidate gene for neuromotor abnormalities. Finally, the two patients displaying both neurological abnormalities and prenatal growth retardation harboured a larger deletion extending telomeric with respect to locus D13S176 (8cM). Thus, our data suggest that contiguous genes centromeric and telomeric with respect to RB1 should account for the neurological impairment and growth retardation in our patients.

**NONSENSE MEDIATED mRNA DECAY FOR hMRE11 MUTATIONS.** *L. Chessa<sup>1</sup>, M. Piane<sup>1</sup>, C. Savio<sup>1</sup>, C. Ciotta<sup>2</sup>, A. Muleti<sup>1</sup>, M. Bignami<sup>2</sup>, G. Giannini<sup>1</sup>, E. Ristori<sup>1</sup>, S. Palmeri<sup>3</sup>, A.M.R. Taylor<sup>4</sup>.* 1) II Faculty Medicine, Hosp S Andrea, Rome, Italy; 2) I.S.S., Roma, Italy; 3) Dept. Neurology, University of Siena, Italy; 4) CRC Institute for Cancer Studies, University of Birmingham, UK.

Brother and sister, born to unrelated healthy parents and now in their thirties, were referred some years ago to the Italian Registry for Ataxia Telangiectasia. They were diagnosed as Ataxia without Telangiectasia, due to the mild and slow-progressive neurological features and the absence of telangiectasias. At cellular level both show intermediate radiosensitivity between classical AT and controls. Lymphoblastoid cell lines were established and subsequently used for molecular studies. No mutations in ATM and NBS genes were found at molecular analysis, performed by means of SSCP, HD and direct sequencing. Western blottings for ATM, NBS and Mre11 proteins showed reduced amounts of the three proteins of the p95/Rad50/Mre11 complex. Sequencing of hMre11 gene allowed the identification of the maternal mutation 1612C>A in compound heterozygosity. The second mutation was not apparent at cDNA level, due to nonsense mediated mRNA decay. In fact, it was a truncating mutation (1714C>T) resulting in a stop codon. Interestingly enough, this second mutation was already found in an English family previously described (Stewart et al., 1999). The functional impact of these mutations on the p95/Rad50/Mre11 complex was analyzed by means of Irradiation Induced Foci. We successfully applied a new protocol to study foci on lymphoblastoid cell lines. The complete abrogation of foci formation after g-irradiation at 12 Gy was observed. Work partially granted by Italian Telethon (E.764), AIRC and COFIN (to L.C.) REFERENCES Stewart et al., 1999. The DNA Double-Strand Break Repair Gene hMre11 is mutated in individuals with an Ataxia Telangiectasia-like Disorder. *Cell* 99,577-587.

**Spectrum and distribution of *FOXL2* gene mutations and variants in BPES, POF and XX male patients: tentative genotype-phenotype correlation.**

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Mutations in *FOXL2*, a forkhead transcription factor gene, have recently been shown to cause type I and II blepharophimosis-ptosis-epicanthus inversus syndrome (BPES). In BPES type I a complex eyelid malformation is associated with premature ovarian failure (POF), while in BPES type II the eyelid defect occurs as an isolated entity. We describe the identification of novel mutations in the *FOXL2* gene in BPES type I and II families, in sporadic BPES patients, and in BPES families where the type could not be established. In 67 percent of the patients studied, we identified a mutation in the *FOXL2* gene. We demonstrate that there is a genotype-phenotype correlation for both types of BPES by the finding that mutations predicted to result in a truncated protein both lacking or containing the forkhead domain lead to type I BPES. In contrast, duplications within or downstream of the forkhead domain, and a frameshift downstream of them, all predicted to result in an extended protein, cause type II BPES. In addition, a series of unrelated patients with isolated POF and XX males were screened for *FOXL2* mutations. Several changes were identified, of which the causality is further investigated. Our study provides further evidence that *FOXL2* haploinsufficiency may cause BPES type I and II by the effect of a null allele and a hypomorphic allele, respectively. Furthermore, we propose that in a fraction of the BPES patients the genetic defect does not reside within the coding region of the *FOXL2* gene and may be caused by a position effect.

**FGFRs and TWIST mutations in craniosynostosis patients.** *C. Carbonara*<sup>1</sup>, *M. Valenzise*<sup>2</sup>, *S. Bosso*<sup>3</sup>, *L. Sbaiz*<sup>3</sup>, *P. Peretta*<sup>4</sup>, *L. Genitori*<sup>4</sup>, *M. Silengo*<sup>2</sup>, *G. Restagno*<sup>3</sup>, *G.B. Ferrero*<sup>2</sup>. 1) Dept Neonatology, OIRM-S.ANNA, Torino, Italy; 2) Dept Pediatrics, Univ Torino; 3) Dept Clinical Pathology; 4) Dept Pediatric Neurosurgery, OIRM-S. Anna, Torino, Italy.

Mutations in three of the four fibroblast growth factor receptor genes (FGFRs) and in the transcription factor TWIST have been reported in several craniosynostosis syndromes. Here we report the results of a screening for FGFRs and TWIST mutations in 90 patients surgically treated for various form of craniosynostosis in our department. Clinically the patients could be classified as follows: Crouzon syndrome (8 cases), Saethre-Chotzen syndrome (7 cases), Pfeiffer syndrome (1 case), Apert syndrome (5 cases), isolated plagiocephaly (18 cases), isolated brachycephaly (8 cases), trigonocephaly (16 cases), scaphocephaly (6 cases) and unclassified forms (21 cases). As already described, we observed a higher percentage of mutations among syndromic patients (75%) compared to isolated cases (26%). No mutations have been detected in patients with trigonocephaly, scaphocephaly and unclassified forms. All FGFR2 mutations observed are known molecular defects and have been found in 6 of the 8 patients, in 1/1 Pfeiffer patient and in 5/5 Apert patients. The recently described FGFR3 Pro250Arg mutation has been observed in 3/7 Saethre-Chotzen, in 3/18 isolated plagiocephaly and in 2/8 isolated brachycephaly. Among the 8 patients carrying this peculiar mutation 5 were familiar and 3 sporadic. Mutations in the TWIST gene have been found in 2/7 Saethre-Chotzen and interestingly in 2 cases of isolated plagiocephaly and brachycephaly respectively. One of these five TWIST mutations have been already reported (R116H), the remaining being newly described. All of them are de novo mutations, involving functional domains of the gene. Due to the wide clinical spectrum of phenotypes associated with these mutations, and in particular with TWIST and FGFR3 Pro250Arg, molecular characterization of these conditions represents an important tool in genetic counselling, clinical classification and in the identification of additional candidate gene.



**Molecular Analysis of Concurrent Williams-Beuren Syndrome and Chronic Granulomatous Disease.** *J.M.*

*Gastier, L. Schulz, J. Frick, A.M. Sommer, G.D. Wenger.* Children's Hosp, Ohio State Univ, Columbus, OH.

Williams-Beuren Syndrome (WBS) and Chronic Granulomatous Disease (CGD) are distinct clinical entities that can be caused by abnormalities of loci in the 7q11.23 region. A patient was identified with both disorders. WBS is caused by a 2-3 Mb deletion of band 7q11.23. CGD is usually an X-linked disorder, but an autosomal recessive form is caused by mutations in the NCF1 gene at 7q11.23. To determine the etiology of WBS and CGD in this patient, molecular genetic analysis was performed on somatic cell hybrids (SCH) with the patient's chromosome 7 homologues separated. The inclusion of NCF1 in the common WBS deletion has remained controversial. Analysis of NCF1 is complicated by the presence of proximal (NCF1P1) and distal (NCF1P2) pseudogenes. Genotype analysis of SCH with D7S489 showed deletion of both D7S489B (proximal to NCF1) and D7S489A (distal to NCF1), suggesting that NCF1 is deleted on one chromosome of this patient. TaqI and PstI digestions were performed on amplicons from exons 3-4 of the NCF1 gene. The PCR primers also amplify the same region in the pseudogenes, but single base differences between the true and pseudogenes were distinguished by the restriction enzymes. Digestions of the product from a SCH with the deleted chromosome 7 confirmed the absence of the NCF1 gene and suggested that NCF1P1 is also deleted. The most commonly observed WBS deletion does not include either the D7S489A or NCF1P1 locus, suggesting that this patient may have a larger WBS deletion than normally observed. The most common mutation in NCF1 that leads to CGD is a two base pair (GT) deletion in exon 2, causing a frameshift. This deletion is present in both pseudogenes. In a normal individual, sequencing is not interpretable after the GT deletion due to a mixed population of gene and pseudogene amplicons. Sequence analysis of SCH and patient peripheral blood suggested the presence of the GT deletion in NCF1 on the nondeleted chromosome, as indicated by clean sequence through the region. CGD in this patient was determined to be caused by deletion of the NCF1 locus on the WBS deletion chromosome and the presence of a GT deletion in exon 2 of NCF1 on the other chromosome.

**New update of the FBN1 mutation database.** *G. Collod-Beroud*<sup>1</sup>, *S. Le Bourdeles*<sup>2</sup>, *I. Dehaupas*<sup>2</sup>, *S. Grey*<sup>2</sup>, *C. Junien*<sup>1, 2</sup>, *C. Beroud*<sup>1</sup>, *C. Boileau*<sup>1, 2</sup>. 1) INSERM U383, Hopital Necker-Enfants Malades, Paris, France; 2) Laboratoire Central de Biochimie, d Hormonologie et de Genetique moleculaire, Hopital Ambroise Pare, AP-HP, Boulogne, France.

A major goal of geneticists is to elucidate the molecular basis of human phenotypes. Approximately 5,000 phenotypes are currently known and the number of genes is estimated to be near 30,000. At present, gene-phenotype associations have been identified only between a few hundred genes and specific diseases. For some genes like FBN1, the gene coding fibrillin-1, the situation is more complex with the association of mutations with a large spectrum of conditions related to Marfan syndrome (MFS)(OMIM 154700). FBN1 has also been shown to harbor mutations in ectopia lentis, neonatal Marfan syndrome, Marfanoid skeletal syndrome, atypical Marfan syndrome without cardiac implication, Shprintzen-Goldberg syndrome, some cases of thoracic aortic aneurysms and probably Weill-Marchesani syndrome. All the mutations described in FBN1 are widely distributed throughout the gene with no apparent disease specific or structural/function relationship. To date, the only genotype/phenotype relationship is still limited to exons 24 to 32 in which most mutations causing neonatal Marfan syndrome are clustered. To facilitate the mutational analysis of FBN1 mutations and the identification of structure/function and phenotype/genotype relationships, we created, in 1995, a human FBN1 mutation database which gives access to a software package that provides specific routines that are optimized multicriteria research and sorting tools [NAR, 1998, vol26: 229-233; Hum Mutation 2000, 15: 86-94]. For each mutation, information is provided at gene, protein, and clinical levels. The database has been recently modified to follow the guidelines on mutation databases of the Hugo Mutation Database Initiative including the new nomenclature [Antonarakis et al., Hum Mutation 1998, 11: 1-3]. The current update shows more than 350 FBN1 (137 mutations in the last publication in 1998) and will soon be available on its Internet Web site. You can access the Marfan Site and Mdb at <http://www.umd.necker.fr/>

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**Myotonic dystrophy in the North of Portugal: a significant but still overlooked problem.** *T. Coelho*<sup>1</sup>, *A. Tuna*<sup>2</sup>, *M. Freijo*<sup>2</sup>, *M.C. Palmares*<sup>3</sup>, *R. Santos*<sup>4</sup>, *M. Santos*<sup>5</sup>, *A. Sousa*<sup>6</sup>. 1) Neurophysiology, HGSA; 2) Neurology, HGSA; 3) Centro Genética Clínica; 4) Inst. Genética Médica Jacinto Magalhães; 5) Neuropediatria, Hospital Maria Pia; 6) Inst. Ciências Biológicas Abel Salazar, Porto, Portugal.

Myotonic dystrophy, one of the most frequent hereditary muscular disorders has never been systematically studied in Portugal. Complete molecular diagnosis became available only recently. Since 1991, 74 cases of myotonic dystrophy from --35 different families were diagnosed at one paediatric (Hospital Maria Pia) and one adult (Hospital Santo António) integrated neuromuscular outpatient clinics in Porto. Recently 32 families (69 patients, 37 female and 32 male) had molecular confirmation of the CTG expansion in chromosome 19. The size of this expansion was quantified in 36 patients. Clinical type, age-of-onset, family history and molecular studies were reappraised in these 69 patients. Age-at-onset was established in 57: congenital form presented in 10 patients (8 probands) and varied from 1 to 63 years in the non-congenital forms. Concerning parental transmission, 28 patients inherited the disease from their fathers and 29 from their mothers; 12 cases had unidentified transmitter. Mean age-of-onset was higher (23.3 years) in offspring of affected fathers than in offspring of affected mothers (13.8 years). Myotonic dystrophy is a relevant problem in the North of Portugal. Systematic molecular diagnosis of all patients will help in ascertainment of families and genetic counselling.

**Diagnostic testing for X-Linked Ocular Albinism (OA1) with an hierarchical mutation screening protocol.** *M.R. Hegde<sup>1</sup>, R.A. Lewis<sup>1,2</sup>, C.S. Richards<sup>1</sup>*. 1) Diagnostic Sequencing Laboratory, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030; 2) Department of Ophthalmology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

Albinism is a group of inherited conditions in which affected individuals have less than normal pigment in the eyes, skin, and hair compared to others of the same race and ethnic background. The prevalence of all types of albinism in the United States is estimated at 1 in 20,000, based on poor epidemiological data. X-linked Nettleship-Falls Ocular Albinism (XLOA or OA1) affects ~ 1/150,000 males in the general population. OA1 results in reduced visual acuity with nystagmus and a mild skin and hair phenotype and occurs mostly in XY males. Female carriers of XLOA/OA1 have normal visual acuity but usually show iris punctate transillumination and a classic mosaic pattern of retinal pigmentation. Studies of OA1 have shown linkage of a single gene to markers at Xp22.3-p22.2. About 48% of the reported mutations in the OA1 gene have been intragenic deletions and about 43% are point mutations. We have developed a hierarchical mutation screening strategy for diagnostic analysis for X-Linked Ocular Albinism with multiplex PCR to detect intragenic deletions, dHPLC (Denaturing High Performance Liquid Chromatography) to scan for mutations, and sequencing to confirm putative mutations in OA1 gene. Prenatal diagnosis can be provided to families after the mutation has been identified. Symptomatic males are tested with a multiplex PCR-based assay for all nine exons of the gene to detect intragenic deletions in the first tier of testing. The second tier of analysis involves the amplification of all exons individually and screening them for point mutations and small deletions/insertions with dHPLC. Variants identified by dHPLC are subsequently sequenced in both directions for mutation/polymorphism identification as defined by ACMG guidelines for interpretation of sequence variations. We have validated this procedure with positive controls that were identified in patients with SSCP. In this hierarchical strategy, these procedures have an analytical sensitivity of >99%.

***MECP2* mutations in atypical patients.** I. Meloni<sup>1</sup>, I. Longo<sup>1</sup>, M. Zappella<sup>2</sup>, A. Renieri<sup>1</sup>. 1) Department of Molecular Biology, Medical Genetics, University of Siena, Siena, Italy; 2) Child Neuropsychiatry, Policlinico Le Scotte, Siena, Italy.

Mutations in the *MECP2* gene cause the severe neurodevelopmental disorder called Rett syndrome. Here we report clinical and mutation analysis of 18 preserved speech variants (PSV). Ten of them, all showing a slow recovery of verbal and praxic abilities, evident autistic behavior and normal head circumference had a *MECP2* mutation (55%).

To test how broad the *MECP2* phenotypic spectrum is, we also screened nineteen girls with a clinical diagnosis of Autism, two of them having lost, along the years, the autistic features reaching a high degree of mental abilities. A pathogenic mutation was found only in the latter two cases (R133C and R453X). These results confirm previous clinical studies suggesting that the spectrum of the *MECP2*-associated phenotype is wide and includes girls with mental abilities considerably higher (I.Q. close to 50) than in classic Rett Syndrome.

Significantly, all mutations found in the atypical cases described above are either missense or late truncating mutations. In particular, we did not find the 4 early truncating hot spots, R168X, R255X, R270X, R294X. These results suggest that early truncating mutations lead always to a poor prognosis (classic Rett) while late truncating and missense mutations lead either to classic Rett or PSV or to girls with an I.Q. close to 50. We hypothesize that a missense or late truncating mutation is necessary but not sufficient to reach a high degree of mental ability, based on the presence of one (or more) modifier genes whose product may interact in an epistatic manner with MeCP2 protein.

**Breakpoint analysis of deletions in patients with Greig cephalopolysyndactyly.** *J.J. Johnston<sup>1</sup>, I.M. Olivos-Glander<sup>1</sup>, J.K. Blancato<sup>2</sup>, L.G. Biesecker<sup>1</sup>.* 1) NHGRI, NIH, Bethesda, MD; 2) Inst. of Mol. Hum. Gen., Georgetown Univ., Washington, D.C.

Greig cephalopolysyndactyly syndrome (GCPS) is due to haploinsufficiency of *GLI3*. The phenotype includes polydactyly, macrocephaly and/or hypertelorism, mental retardation (MR), hernias and dysgenesis of the corpus callosum. A range of genetic alterations affecting *GLI3* is responsible for GCPS including point mutations, frameshifts, and deletions. We have analyzed 28 families for deletions of 7p13. Ten of our families have deletions and we are working to identify the breakpoints to determine if a common mechanism underlies the deletions in GCPS. Furthermore, identifying the breakpoints will allow us to correlate clinical findings in our families with their complement of haploinsufficient genes. Initial analysis has shown developmental delay (DD) and MR to be more prevalent in deleted families (9/10) than in undeleted families (1/13) and we hypothesize that this is due to other genes in the region. STS analysis of hybrids with only the deleted chromosome 7 from each patient is being used to determine the extent of the deletions. We currently have hybrid clones from 4 patients and have determined the maximum deletion sizes, which range from 180 Kb to 12 Mb. Patient G-2, the only deleted individual that does not have DD or MR, has a deletion restricted to *GLI3*. The deletion breakpoints are within BACs AC005026 and AC005158. The deletion in patient G-11, diagnosed with DD, deletes *GLI3* and *INHBA* but no other known genes. The breakpoints are in BACs AC012596 and AC073181. Patients G-17 and G-21 have larger deletions including more than 30 genes. The telomeric breakpoint in G-17 lies in BAC AC005483 and the centromeric breakpoint lies between BACs AC004859 and AC069282. The telomeric breakpoint in G-21 is just telomeric to BAC AC005483 and the centromeric breakpoint lies between BACs AC011992 and AC004920. Results from FISH and STRP analysis show an additional 4 patients with deletions larger than *GLI3*. Importantly, all of these breakpoints are distinct. We conclude that *GLI3* deletions associated with GCPS have unique breakpoints and are not generated by stereotypical deletions.

**The Spectrum of Glucocerebrosidase Mutations among Korean Gaucher pts: its implications on clinical phenotype and genetic diversity.** *H.J. Kim<sup>1,2</sup>, C.A Yang<sup>2</sup>, S.J. Park<sup>2</sup>, M.J. Ha<sup>2</sup>, W.K. Kim<sup>3</sup>*. 1) Genetics Clinic, Ajou Univ. Hosp; 2) Medical Genetics Research lab, Ajou Univ. Sch. of Med; 3) Lab of Molecular biology, Ajou Univ. Sch. of Med.

Mutational analysis of glucocerebrosidase(GC) gene in 20 unrelated Korean Gaucher Disease pts(9 type1, 4 type2, 4 type3A, 3 type3B) identified 12 different mutations in 27 alleles by direct sequencing of PCR amplified genomic DNA. L444P mutation was found to be most prevalent (20%) in both non-neuronopathic(NNP) and neuronopathic (NP) phenotypes. Next common mutation, F213I was found in one allele of type 1 & 3 alleles of NP. R257Q, a rare mutation was also detected in both phenotypes for one each. G46E was detected only in NNP and G202R only in NP (3 alleles for each). Other 7 rare mutations were identified in only one allele for each mutation (R48W&V406V in type1 and N188S, V191G, F331L, G377S and D409H in NP). The lack of N307S mutation, known to be associated with a mild type1 phenotype and protective mutation from neuronopathic phenotype among Korean may explain the phenotypic characteristics of non-neuronopathic type1 being associated with early onset in childhood & severe phenotype and the preponderance of neuronopathic phenotype(>50%)among Korean GD pts. L444P, common mutation (about40%)among Non-jewish Caucasian and Japanese, is known to be associated with neuronopathic phenotype, and esp. homoalleles for L444P is most common in type 3 pts. However among 11 Korean neuronopathic pts. only 3 alleles (< 15%) and no homoalleles were detected. Instead, two homoalleles for rare mutations, G202R in type1 and F213I in a type3B ,were found. V191G, previously reported as a novel mutation of mild phenotype in type1 was detected in a heteroallele with R257Q in a type2 pt. The spectrum of 12 identified GC mutations among Korean is rather unique from those of Jewish & non-Jewish Caucasian, but similar to that of Japanese, yet the striking difference in L444P homoallelic frequency and other allelic heterogeneity exist between GD, especially neuronopathic form of Korean & Japanese, which can be explained in part with genetic diversity and cultural difference toward consanguinity mating.

**Increased diagnostic and prognostic sensitivity of spinal muscular atrophy (SMA) testing.** *M.D. Mailman<sup>1</sup>, J.W. Heinz<sup>1</sup>, B. Wirth<sup>2</sup>, A.H.M. Burghes<sup>3</sup>, T.W. Prior<sup>1</sup>.* 1) Dept Pathology, Ohio State Univ, Columbus, OH; 2) Institute of Human Genetics, Bonn, Germany; 3) Dept of Medical Biochemistry, Ohio State University, Columbus, OH.

Spinal muscular atrophy (SMA) is an autosomal recessive hereditary neuromuscular disease characterized by proximal muscle weakness due to the loss of a-motor neurons in the spinal cord. In very well characterized SMA cases, the survival motor neuron (SMN1) gene on chromosome 5q13 is homozygously deleted more than 95% of the time. The remaining patients have a neurodegenerative condition unassociated with chromosome 5q or are compound heterozygotes with an intragenic mutation and a deletion. To increase the sensitivity for detection of mutations in the SMN1 gene, we have developed an allele-specific fluorescent PCR panel that detects 7 of the most common intragenic mutations found in SMA patients. Identification of an intragenic mutation is followed by a dosage test to confirm compound heterozygosity. Results of a SMN2 gene dosage study comparing 52 type I versus 90 type II and III SMA patients indicated that patients with a more mild presentation have significantly more copies of the SMN2 gene than severe cases of the disease. We present data comparing ages at diagnosis with SMN2 copy number. Finally, we present results of five years of SMA carrier testing. Since we began offering the SMN1 dosage test in 1996, our laboratory has tested 399 family members for SMN1 deletion carrier status. The results of these carrier tests are consistent with SMA segregating with a carrier frequency of 3% and a combined new mutation or two-copy chromosome rate of 5%.a.



**Genotype/phenotype correlations in 202 Rett syndrome patients.** *L.M. Moses<sup>1</sup>, K.C. Hoffbuhr<sup>1</sup>, C. Scacheri<sup>1</sup>, L. Falke<sup>1</sup>, S. Budden<sup>2</sup>, A. Percy<sup>3</sup>, M. Philippart<sup>4</sup>, G. Bibat<sup>5</sup>, S. Naidu<sup>5</sup>, E.P. Hoffman<sup>1</sup>.* 1) Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) Child Development and Rehabilitation Center, Oregon Health Sciences University, Portland, OR; 3) Pediatric Neurology, University of Alabama at Birmingham, Birmingham, AL; 4) Mental Retardation Research Center, UCLA, Los Angeles, CA; 5) Neurogenetics Unit, Kennedy Krieger Institute, Johns Hopkins University, Baltimore, MD.

Rett syndrome (RTT) is a neurodevelopmental disorder in girls caused by mutations in the methyl CpG binding protein 2 (*MeCP2*). We performed mutation analysis of the *MeCP2* gene in 202 individuals with variable clinical diagnoses. In this study, we identified a total of 37 different mutations, including 10 novel mutations. Those with a typical presentation of RTT showed a high percentage of *MeCP2* mutations (85%; 68/80). In contrast, only 25% (7/28) of individuals with a diagnosis of atypical RTT had mutations in the *MeCP2* gene. *MeCP2* mutations were not detected in 10 males with variable clinical presentation or 21 individuals with uncharacterized neurological disorders and no specific diagnoses. Clinical information was not available for 63 patients; 34 mutations were identified in these individuals (54%). Eight mutations (R106W, R133C, T158M, R168X, R255X, R270X, R294X, R306C) accounted for 72% (49/68) of all mutations in those with classical RTT and 57% (4/7) in those with atypical RTT. Of these 7 individuals, 3 showed frame-shift deletions in the C-terminal of *MeCP2*. We identified 2 novel rearrangements in the C-terminus of the *MeCP2*: the first involving two in-frame deletions (93bp and 18bp) and an 8bp out of frame insertion, and the second involving a 211bp out of frame deletion and a 111bp in-frame inversion. This study identified several novel polymorphisms in the *MeCP2* gene, including a 12 basepair in frame deletion that results in the loss of 4 amino acids in a non-conserved C-terminal region of the *MeCP2* gene.

**Screening of Pancreatic Secretory Trypsin Inhibitor (PSTI) mutations in chronic pancreatitis by DHPLC.** *C. Le Marechal<sup>1</sup>, O. Raguene<sup>2</sup>, I. Quere<sup>2</sup>, J. Chen<sup>1</sup>, C. Ferec<sup>1,2</sup>*. 1) EFS Bretagne, Brest, France; 2) CHU de BREST, France.

Over the past five years, genetic studies of patients with Chronic Pancreatitis (CP) have been led in order to find an etiology in idiopathic forms of CP. "Gain of function" missense mutations in the cationic trypsinogen gene have been increasingly identified as being associated with hereditary and/or sporadic pancreatitis. Based upon a candidate approach, presumably "loss-of-function", mutations in the pancreatic secretory trypsin inhibitor (PSTI) gene can be involved CP disease. A N34S missense mutation, was identified as being strongly associated with chronic pancreatitis in young patients. We extended to evaluate the PSTI gene in a large cohort of unrelated French subjects with CP by Denaturing High Performance Liquid Chromatography (DHPLC). The major new findings are as follows. Firstly, we confirmed that N34S carrier frequency in the < 20 years group is significantly higher than the 25~65 years group, suggesting an increased environmental contribution to the disease in the later group. Secondly, in addition to other known variants (e.g., c.-53C>T and c.-41G>A), five novel variants were identified (-2C>A, L12F, L14R in exon 1 ; IVS2+54C>T in exon 2 ; R65Q in exon 3). The L12F variant was found in three unrelated CP patients and none control. It thus can probably be considered as a disease associated nucleotide change. The R65Q variant was found in only one control and is probably a polymorphism. Others were discovered only once in CP patient, so their interpretation remain difficult. This study confirmed the potential of DHPLC to screen quickly and accurately a gene and could be used for scanning others candidate genes implicated in CP. These results help further resolve the elusive etiology of sporadic pancreatitis.

**A new family with a mutation in exon 2 of COL2A1 and Stickler syndrome without systemic manifestations.** *A.H. van der Hout<sup>1</sup>, E. Verlind<sup>1</sup>, H. Scheffer<sup>1</sup>, J.M.M. Hooymans<sup>2</sup>*. 1) Dept Clinical Genetics, University Hospital Groningen, Groningen, Netherlands; 2) Dept Ophthalmology, University Hospital Groningen, Groningen, Netherlands.

Stickler syndrome is an autosomal dominantly inherited connective tissue disorder with ocular, orofacial, auditory and articular manifestations. There are two types, based on different abnormalities in the vitreous gel. Type 1 families show a characteristic membranous anomaly in the vitreous gel. In these families mutations are found in the COL2A1 gene. In some families with the type 1 vitreous phenotype there are no systemic manifestations of the disease. Richards et al. (2000) found mutations in exon 2 of COL2A1 in 3/3 families with the type 1 vitreous phenotype and almost no systemic manifestations. This is consistent with the major form of type II procollagen in non-ocular tissues having exon 2 spliced out. All three mutations resulted in a premature stopcodon in exon 2. In our family linkage to COL2A1 was established several years ago. There are 25 affected individuals, all with the characteristic type 1 abnormal vitreous gel. No systemic signs of Stickler syndrome, such as joint hypermobility, high arched or cleft palate, abnormalities of the dentition or craniofacial skeleton, or hearing loss, are present. Sequencing of exon 2 of COL2A1 revealed an A to C mutation, changing codon 64 into a stopcodon. This mutation is different from the three described by Richards et al. Sequencing of exon 2 of COL2A1 can be a quick method to find a mutation in families with autosomal dominant vitreoretinal degeneration, even if the family is too small for linkage analysis. In counselling it is important to realise that individuals with an exon 2 COL2A1 mutation are at high risk of retinal detachment, but at a much lower risk of developing the other features of Stickler syndrome. Richards et al., *Br. J. Ophtalm.* (2000) 84, 364-371.

**Nested deletions in the murine region syntenic to the human Smith-Magenis syndrome.** *J. Yan*<sup>1</sup>, *S. Carattini-Rivera*<sup>1</sup>, *K. Walz*<sup>1</sup>, *A. Bradley*<sup>2</sup>, *J.R. Lupski*<sup>1</sup>. 1) Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX; 2) Sanger center, Cambridge, UK.

Smith-Magenis syndrome (SMS) is a microdeletion syndrome on chromosome 17p11.2. The main clinical manifestations include mental retardation, developmental delay, craniofacial anomalies, neurobehavioral abnormalities, and defects of heart and kidneys. The complex phenotype is thought to result from haploinsufficiency effects of contiguous genes that map in the critical interval. Because there are no small deletions in the patients that enable us to elucidate the gene or genes responsible for different phenotypes, we began to construct nested deletions in murine syntenic region to the human SMS common deletion. The murine syntenic region maps to the chromosome 11 32-34 CM interval. Many genes mapped to the human SMS region also have been mapped to the murine syntenic region. In order to make nested deletions, we first targeted *Cops3*, a gene at one end of the murine syntenic region, with one loxP and half of the *Hprt* gene. Another loxP site and another half of the *Hprt* gene were introduced using an engineered retrovirus. After recombination between two loxP sites by Cre recombinase, we obtained 39 deletion clones. Southern-blot analysis of the proviral junction fragments demonstrates that there are ten independent deletion events. FISH and inverse PCR(IPCR) have been carried out to identify the deletion end points. ES cells are now waiting injection. Our work will lead us to the gene or genes responsible for different phenotypes, and will eventually enable an understanding of the molecular basis for the SMS.

**Identification of novel CLCN1 and SCN4A mutations in a clinical spectrum of ion channelopathy patients.** *F.F. Wu*<sup>1,2</sup>, *J. Devaney*<sup>3</sup>, *T. Hodics*<sup>4</sup>, *Z. Korade-Mirnic*<sup>5</sup>, *E. Pegoraro*<sup>5</sup>, *J. Giron*<sup>2</sup>, *D. Escolar*<sup>2</sup>, *M. Marino*<sup>3</sup>, *E.P. Hoffman*<sup>2</sup>.  
1) Human Genetics, University of Pittsburgh, PA; 2) Children's National Medical Center, Washington, DC; 3) Transgenomic Inc, Gaithersburgh, MD; 4) George Washington University, Washington, DC; 5) Department of Molecular Genetics and Biochemistry, University of Pittsburgh, PA.

The muscular chloride and sodium channel disorders (channelopathies) represent a group of clinically overlapping and genetically heterogeneous diseases showing abnormal alterations in the excitability of the sarcolemma. The cardinal symptoms include non-dystrophic and non-progressive myotonia and periodic paralysis. Over the past decade, most efforts to elucidate the genetic causes and pathophysiologic features of myotonia and paralysis have been focused on the typical phenotypes, reducing the potential of identifying new mutations that may result in unusual phenotypes. In this study, we widened our clinical window to analyze a broad spectrum of myotonia and paralysis phenotypes. We used a cohort of patients referred from molecular diagnosis to be negative for common mutations of CLCN1 and SCN4A. We screened all exons of CLCN1 in 115 familial and sporadic myotonia patients and all exons of SCN4A in 56 periodic paralysis patients. SSCP and DHPLC were used for screening, followed by direct sequencing. We identified six novel CLCN1 mutations (five missense and one nonsense) and five new missense SCN4A mutations. While the former were shown to be distributed all over the chloride channel protein, the latter resided in unusual regions of the sodium channel protein (amino and carboxy termini), which could account for the atypical phenotypes. Our study not only identified significant number of novel CLCN1 and SCN4A mutations but also proved that the clinical variability and genetic heterogeneity of channelopathies is larger than commonly believed, and that there are broader and atypical myotonia and periodic paralysis yet to be characterized in which the causative mutations of the condition reside equally in the CLCN1 or SCN4A. These unexpected results open a new pathway to further characterization of the important group of channelopathies.

**The I148T *CFTR* mutation confers a variable phenotype and occurs on multiple haplotypes.** *E.M. Rohlf's*<sup>1</sup>, *Z. Zhou*<sup>2</sup>, *E.A. Sugarman*<sup>1</sup>, *R.A. Heim*<sup>1</sup>, *R.G. Pace*<sup>3</sup>, *M.R. Knowles*<sup>3</sup>, *L.M. Silverman*<sup>2</sup>, *B.A. Allitto*<sup>1</sup>. 1) Genzyme Genetics, Framingham, MA; 2) University of North Carolina, Dept Pathology & Laboratory Medicine; 3) University of North Carolina, Dept Medicine, Chapel Hill, NC.

The cystic fibrosis (CF) phenotype varies from mild to severe disease and is in part determined by the *CFTR* genotype of the individual. For example, the phenotypic effect of the R117H mutation is modified by the length of the polythymidine tract (5, 7 or 9 thymidines) found in intron 8. The I148T mutation has been characterized as "severe" due to its identification in pancreatic insufficient CF patients. However, we have evidence from carrier testing that the I148T mutation is associated with a variable phenotype. We identified 5 unaffected individuals (ages 21 to 56) with a DF508/I148T genotype who had been referred for carrier testing. The presence of both mutations on the same chromosome was ruled out by family studies in 2 individuals. Carrier testing of 42,784 individuals of varying ethnic backgrounds identified 1754 *CFTR* mutation carriers and I148T accounted for 6.4% of the mutations identified. Yet, this mutation accounts for only 0.06% of the mutations in individuals whose indication for testing was "known affected with CF" (n=9236 chromosomes). To determine if other changes in the *CFTR* gene were modifying the CF phenotype, we sequenced all *CFTR* exons and the intron 8 polythymidine tract from 5 reportedly unaffected individuals (genotypes I148T/I148T or DF508/I148T) and from 5 individuals with a known or suspected diagnosis of CF (genotypes DF508/I148T or N1303K/I148T). We have determined that the I148T mutation occurs on at least 2 haplotypes: 1) a 9T background which was identified in all 5 unaffected individuals and 1 affected individual 2) a 9T/3199del6 background which was identified in 4 of the 5 affected/suspected individuals. The 3199del6 sequence change was not identified in 386 non-CF chromosomes. We conclude that I148T occurs on at least 2 haplotypes and these haplotypes may differ between those individuals with and without symptoms of CF.

**Mutation analysis in the noncoding region of *ABCA4* (promoter region and genomic rearrangements) among STGD patients.** A.N. Yatsenko, N.F. Shroyer, R.A. Lewis, J.R. Lupski. Human Molecular Genetics, Baylor College Med, Houston, TX.

Stargardt disease (STGD1, OMIM#248200) is an autosomal recessive retinal dystrophy classically observed at an early age. The estimated frequency of STGD1 in the United States is between 1:8,000-10,000. The causative gene for STGD1 has been cloned in the chromosome region 1p21-1p13. This gene, *ABCR* (also known as *ABCA4*), contains 50 exons and encodes a photoreceptor-specific ATP-binding cassette transporter, which expresses specifically in the both cone and the rod photoreceptors. *ABCR* protein appears to function as an outwardly directed flippase for retinoids. Previous studies showed a range of different types of mutant *ABCR* alleles in STGD1 families. Recently, based on direct DNA sequencing of all *ABCR* exons, we have performed mutation screening for patients with late-onset STGD1 (Yatsenko et al. 2001) and STGD patients with AMD relatives (Shroyer N.F., personal data). We found mutations in 66% and 80% of disease chromosomes. Therefore we suggested that a potential cause for the remaining 34-20% STGD alleles are noncoding *ABCR* mutations or other gene(s) located in distinct chromosome regions. To investigate the disease cause in these patients we analyzed the promoter region, which spans ~3 kb located 5' to transcription start and includes exon 1. Based on published prediction for 0.8 kb 5' region and our computer analysis we predict several potential sites for transcription factors. We performed direct sequence of the ~3 kb region in STGD patients. We identified several potential mutation sites involved in transcription regulation. Direct sequencing of same region in control individuals is in progress. To further test these potential sites we will perform a functional reporter assay for them. These functional assays will be performed in retina-specific lines Y79 and WERI, which express several photoreceptor specific proteins. In addition to test the possibility of DNA rearrangement mutations in the same patients we performed Southern analysis with *ABCR* gene specific probes. Preliminary data showed few STGD patients with potential junction fragments in *ABCR* specific region. Further analysis will confirm or refute pathogenic role of these findings.

**Bloom's syndrome: Cellular BLM proteins from splice-site mutations.** *M.M. Sanz*<sup>1</sup>, *R.M. Pauli*<sup>2</sup>, *N.A. Ellis*<sup>3</sup>, *J. German*<sup>1</sup>. 1) Cornell University Medical College, New York, NY; 2) University of Wisconsin, Madison, WI; 3) Memorial Hospital, New York, NY.

Of the 64 distinct Bloom's syndrome (BS)-causing mutations we have characterized at the molecular level, 9 are altered splice sites. Cultured cells from a sub-set of these 9, affecting splice-donor sites in introns 2, 6, 7, and 18, were studied by immunofluorescence microscopy employing antibodies to BLMp and PMLp. Proliferating normal cells have abundant nuclear BLMp, distributed as diffuse granularity or concentrated in foci, some foci appearing as extremely fine "speckles" and a few as larger "dots," many of the dots co-localizing with PML nuclear bodies. In all four splice-site mutant cell lines, nuclear fluorescence is demonstrable by antiBLM in some but not all cells, the amounts (always very low) and distributions being distinctive in each of the four. The diffuse granularity seen in normal cells is greatly reduced or undetectable. BLM foci are produced in each splice-site mutant, but with respect to number, size, and degree of co-localization with PML nuclear bodies the mutant cells differ from normal, and also from each other. In 2 mutants, PML bodies are unusually numerous, large, and irregular in contour.

One mutation, IVS6+3A->G, well upstream of the recQ helicase-encoding regions of *BLM*, is of special interest because the affected young man, 112(NaSch) in the Bloom's Syndrome Registry, has a relatively normal phenotype (re height, testicular size, immunocompetence). He is a genetic compound whose second *BLM* mutation, A814T, is truncating. The abnormal but possibly functional BLM protein(s) that 112(NaSch)'s cells synthesize by some undefined splicing pattern now can be characterized extensively at the molecular level. We postulate that they (i) can carry out some of BLMp's normal functions, perhaps the control (suppression) of recombination in those cellular mechanisms concerned with body-size determination, immune function, and germ-cell maturation but, because his SCE rate is characteristically elevated, (ii) cannot control recombination during S-phase DNA synthesis in somatic cells.



**Erythrocyte Ankyrin Promoter Mutations Associated With Recessive Hereditary Spherocytosis Cause Significant Abnormalities in Ankyrin Expression.** *P.G. Gallagher<sup>1</sup>, D.E. Sabatino<sup>2</sup>, D.M. Nilson<sup>2</sup>, C. Wong<sup>1</sup>, A.P. Cline<sup>2</sup>, L.J. Garrett<sup>2</sup>, D.M. Bodine<sup>2</sup>.* 1) Dept Pediatrics, Yale Univ Sch Medicine, New Haven, CT; 2) Hematopoiesis Section, NHGRI, NIH, Bethesda, MD.

Ankyrin deficiency is the most common abnormality found in the erythrocyte membranes of patients with hereditary spherocytosis (HS). Several reports have identified sequence variations in the ankyrin gene promoter in individuals with recessively inherited, ankyrin-deficient spherocytosis. These variations have been proposed to decrease ankyrin synthesis, but there are no functional data to support this hypothesis. We analyzed the effects of two common ankyrin gene erythroid promoter mutations, -108T-C and -108T-C *in cis* with -153G-A, on ankyrin expression. No difference between wild type and mutant promoters was demonstrated in reporter gene transfection or gel shift assays *in vitro*. Transgenic mice with a wild type ankyrin promoter linked to a human <sup>A</sup>g-globin gene expressed g-globin in 100 percent of erythrocytes in a copy number-dependent, position-independent manner. Transgenic mice with the mutant -108 promoter demonstrated variegated g-globin expression, but showed copy number-dependent and position-independent expression similar to wild type. Severe effects in ankyrin expression were seen in mice with the linked -108/-153 mutations. Two lines did not express the transgene, indicating position-dependent expression, and 5 lines expressed significantly lower levels of <sup>A</sup>g-globin mRNA than wild type (0.006 percent vs. wild type 0.041 percent, *p* less than 0.0001). Three of 5 expressing lines showed variegated g-globin expression and there was no correlation between transgene copy number and RNA level, indicating copy number-independent expression. These data are the first demonstration of functional defects due to HS-related, ankyrin gene promoter mutations. These promoter mutations may represent a common pathogenetic mechanism of recessive hereditary spherocytosis in ankyrin-deficient patients.

**Genetic heterogeneity of autosomal dominant nonsyndromic macrothrombocytopenias.** *A. Savoia*<sup>1</sup>, *C.L. Balduini*<sup>2</sup>, *M. Savino*<sup>3</sup>, *P. Noris*<sup>2</sup>, *A. Bianco*<sup>1</sup>, *M. Del Vecchio*<sup>3</sup>, *S. Perotta*<sup>4</sup>, *M. Di Pumpo*<sup>2</sup>, *V. Poggi*<sup>5</sup>, *L. Zelante*<sup>3</sup>, *A. Iolascon*<sup>6</sup>. 1) Telethon Institute of Genetics and Medicine (TIGEM), Naples; 2) Department of Internal Medicine, University of Pavia, Pavia; 3) Medical Genetic Service, IRCCS Hospital CSS, San Giovanni Rotondo, Foggia; 4) Department of Pediatrics, II University of Naples, Naples; 5) Department of Pediatric Hematology, Azienda Santobono, Pausilipon, Naples; 6) Department of Biomedicine of Evolutive Age, University of Bari, Bari, Italy.

We have studied an autosomal dominant macrothrombocytopenia characterized by mild or no clinical symptoms, normal platelet functional activity, and normal megakaryocyte. Linkage analysis in two large families localized the gene on chromosome 17p, in an interval containing the GPIIb gene, which is altered in Bernard-Soulier syndrome (BSS). A heterozygous Ala156Val missense substitution (Bolzano variant) was identified in the two families and in another six pedigrees. BSS is an autosomal recessive disorder characterized by prolonged bleeding time, thrombocytopenia, and large platelets due to a defect of the platelet glycoprotein (GP) Ib/IX/V complex. GPIb/IX/V consists of four genes, GPIIb, GPIIb3, GPIIX, and GPV. BSS patients are homozygotes or compound heterozygotes for mutations in these genes. Consistent with a BSS heterozygous condition, the vWF receptor GPs were reduced in all patients with the Bolzano variant. Platelet membrane GP studies were performed on families characterized by macrothrombocytopenia without Ala156Val. The analysis distinguished two groups: 3 patients had the GPIb/IX/V complex normally distributed (true autosomal dominant macrothrombocytopenia) and 5 patients showed a reduction of GPs. We hypothesized that mutations in the BSS genes were responsible for the phenotype of the second group. Thus, the coding regions of the GPIb/IX/V complex genes were amplified. Sequencing analysis of the PCR product excluded the presence of mutations. These results suggest that there is at least another gene responsible for the BSS heterozygous phenotype. A positional cloning strategy based on linkage analysis and mutation screening in candidates is in progress.

**Identification of the gene for Oral-facial-digital type I syndrome (OFD1).** *B. Franco<sup>1</sup>, M.I. Ferrante<sup>1</sup>, G. Giovanna<sup>1</sup>, S. Feather<sup>2,3</sup>, A. Bulfone<sup>1</sup>, V. Wright<sup>2</sup>, A. Selicorni<sup>4</sup>, F. Scolari<sup>5</sup>, A. Woolf<sup>3</sup>, S. Odent<sup>6</sup>, B. Le Marec<sup>6</sup>, S. Malcom<sup>2</sup>, R. Winter<sup>2</sup>, A. Ballabio<sup>1</sup>.* 1) TIGEM, Telethon Inst Genet&Med, Naples, Italy; 2) Insitute of Child Health, Clinical and Molecular Genetics Unit, London UK; 3) Insitute of Child Health, Nephro-Urology Unit, London UK; 4) Clinica Pediatrica De Marchi, Milan, Italy; 5) Division of Nephrology, Spedali Civili and University of Brescia, Italy; 6) Department of Pediatrics and Genetics, University Hospital Pontchaillou, Rennes, France.

Oral-facial-digital syndromes (OFDS) are a heterogeneous group of developmental disorders of which at least nine different forms have been described. Oral-facial-digital type 1 syndrome (OFD1; MIM 311200) is transmitted as an X-linked dominant condition with lethality in males and is characterized by malformations of the face, oral cavity, and digits with a highly variable expressivity even within the same family. Malformation of the brain and polycystic kidneys are commonly associated with this disorder. The locus for OFD1 was mapped by linkage analysis to a 12 Mb interval flanked by markers DXS85 and DXS7105 in the Xp22 region. To identify the gene responsible for this syndrome we analyzed several transcripts mapping to the region and found mutations in OFD1 (formerly named Cxorf5/71-7a) encoding a protein containing coiled-coil  $\alpha$ -helical domains. Seven patients affected by OFD1 were analyzed including three familial and four sporadic cases. Analysis of the familial cases revealed a missense mutation, a 19-bp deletion and a single base pair deletion leading to a frameshift. In the sporadic cases we found a missense (de novo), a nonsense, a splice and a frameshift mutation. RNA in situ studies on mouse embryo tissue sections show that *Ofd1* is developmentally regulated and is expressed in all tissues affected in OFD1 syndrome. The involvement of OFD1 in oral-facial-digital type 1 syndrome demonstrates an important role of this gene in human development.

**Identification and mutation screening of candidate genes for X-linked cleft palate and ankyloglossia (CPX). C.**

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Cleft palate has an incidence in humans of approximately 1/1500. It most commonly occurs sporadically and displays a multifactorial mode of inheritance. The complex interactions between environmental and genetic factors have made it difficult to identify the genetic defects responsible for clefting. However, several families from different ethnic origins have been reported in which cleft palate displays an X-linked, semi-dominant mode of inheritance. Ankyloglossia (tongue-tie) has also been reported in most of these families, and along with submucous cleft and bifid or absent uvula this has become an important diagnostic criteria for X-linked cleft palate (CPX, MIM 303400). Linkage data performed on five well characterised families localises the gene responsible for CPX to Xq21, between the markers *PGK1* and *DXS1217*. This genomic region has been examined for candidate genes by analysis of genomic sequence using gene prediction programmes or using the Human Genome Project (HGP) web site (<http://genome.cse.ucsc.edu/>). The current transcript map contains approximately 15 genes. The full genomic structure of candidate genes has been determined, to allow for mutation detection by direct DNA sequencing. Here we present the transcript map and sequence analysis of candidate genes in 6 CPX families.

**Naxos disease: Linkage analysis and evidence for genetic heterogeneity.** *K. Djabali<sup>1</sup>, A. Martinez-Mir<sup>1</sup>, L. Horev<sup>3</sup>, L. Klapholtz<sup>3</sup>, B. Glaser<sup>4</sup>, A. Zlotogorsky<sup>3</sup>, A.M. Christiano<sup>1,2</sup>.* 1) Department of Dermatology, Columbia University, New York, NY; 2) Department of Genetics and Development, Columbia University, New York, NY; 3) Department of Dermatology, Hadassah Medical Center, Jerusalem, Israel; 4) Department of Endocrinology, Hadassah Medical Center, Jerusalem, Israel.

Naxos disease is an autosomal recessive syndrome affecting the heart, skin and the hair. The heart disease, right ventricular dysplasia (ARVD), is severe and may result in arrhythmia and premature sudden death. The patients also present with nonepidermolytic keratoderma, mainly involving pressure areas in the palms and soles. Finally, the hair involvement is unique, and is characterized by woolly, curly, rough and light colored scalp hair and sparse eyebrows. A similar triad of symptoms has been previously described in patients from the Greek island of Naxos and from Ecuador, with combined epidermolytic palmoplantar keratoderma with woolly hair and dilated cardiomyopathy. Recently, mutations in the plakoglobin and desmoplakin genes have been identified as responsible for the disease in the Greek and Ecuadorean families, respectively. Here we report two new consanguineous families with Naxos disease from Israel. We first excluded a mutation in the desmoplakin and plakoglobin genes by sequence and cosegregation analysis, suggesting that at least one additional gene underlies Naxos disease in these families. On the basis of the role of both desmoplakin and plakoglobin in cell adhesion, we analyzed several other genes coding for components of the desmosomes or proteins involved in different aspects of cell adhesion. These included the genes coding for type I and type II keratins, on chromosomes 17 and 12, respectively, desmoyokin, on 11q13.1, the desmocollin/desmoglein cluster, on 18q12.1, plakophilin 1, on 1q32, plakophilin 2, on 12p13, and plakophilin 4, on 2q23-q31. None of these regions showed cosegregation with the disease trait or homozygosity in these inbred families. According to these results, we are currently performing a genome-wide search to identify the gene underlying the particular presentation of Naxos disease in the families studied.

**Novel mutations and polymorphism of CYP1B1 in Ecuadorian patients with congenital glaucoma.** *S.M. Curry*<sup>1</sup>, *A.G. Daou*<sup>1</sup>, *A. Molinari*<sup>2</sup>, *R.A. Lewis*<sup>1,3,4,5</sup>, *B.A. Bejjani*<sup>1</sup>. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Hospital Metropolitano, Quito, Ecuador; 3) Ophthalmology, Baylor College of Medicine, Houston, TX; 4) Medicine, Baylor College of Medicine, Houston, TX; 5) Pediatrics, Baylor College of Medicine, Houston, TX.

Primary congenital glaucoma (PCG) is an autosomal recessive developmental anomaly of the anterior chamber that manifests in the perinatal or infantile period. It is the most common form of glaucoma in infancy. Mutations in the cytochrome P4501B1 gene (*CYP1B1*) have been shown to cause the disease in about 80 percent; of patients with PCG. More than seventeen different *CYP1B1* mutations have been reported in PCG families from the US, Turkey, Saudi Arabia, Canada, UK and Japan. There is anecdotal evidence that PCG is common in the Native American population of Ecuador. We studied fifteen such families from Ecuador by PCR amplification of the *CYP1B1* coding exons followed by sequencing of the PCR products. Individuals in whom no mutations were detected by PCR amplification and direct sequencing were studied by Southern blot analysis to investigate the possibility of large rearrangements not detectable by the PCR method. These studies identified a compound heterozygous mutation in an affected individual (G61E/4340delG) and a novel homozygous missense mutation in another affected individual (R390C). Furthermore, a novel silent nucleotide polymorphism at position 563 from the start site (C to A) was also found in a family in whom no PCG-associated mutations were detected. No mutations were found in 13 out of the 15 families studied. Southern blot analysis with 3 restriction enzymes on patients and normal controls did not identify any genomic rearrangements of *CYP1B1*. These results show that mutations in *CYP1B1* are a cause of PCG in a small percentage of patients in Ecuador and suggest that there is at least one additional locus yet to be identified. Finally, these studies show that there are individuals and families with unique or rare sequence polymorphisms in addition to the known common polymorphisms already described in *CYP1B1*.

**Identification, characterization and testing of candidate genes for the CDB2 form of autosomal dominant corneal dystrophy located on chromosome 10q23-25.** *S.J. Bowne<sup>1</sup>, X. Xu<sup>1</sup>, A.R. Carr<sup>2</sup>, S.P. Daiger<sup>2</sup>, R.W. Yee<sup>1</sup>.* 1) Ophthalmology & Visual Sciences, Univ. of Texas Health Science Center, Houston, TX; 2) Human Genetics Center, Univ. of Texas Health Science Center, Houston, TX.

Corneal dystrophy affects thousands of Americans and is characterized by irregular scarring of the epithelium, Bowman's membrane, or stroma. Irregular scarring of these layers of the cornea leads to progressive visual impairment, often associated with recurrent attacks of irritation, pain, and photophobia. To date, mutations in seven genes have been associated with corneal dystrophy, but the genes and mutations associated with 5 additional corneal dystrophy loci remain to be identified. One uncloned loci, CDB2, has been mapped to chromosome 10q23-q25 using a large American family. In order to elucidate the gene and mutation associated with this disease, we created a gene map of the minimal disease region on chromosome 10 using information contained in both the public and private human genome databases. This map contains over 100 candidate genes and EST clusters. These genes and EST clusters were prioritized as candidates for disease based upon positive expression in the cornea, biological relevance to disease, and the availability of overlapping genomic sequence. To date, three genes, glutathione-S-transferase-like mRNA (GSTTLp28), stearoyl-coA desaturase (SCD), and actin-related protein 1 (ACTR1A) have been characterized and tested. The genomic structure of each gene was determined and then one affected and one unaffected member of the CDB2 family were tested for the presence of disease-causing mutations by automated PCR product sequencing. No variants were detected in any of the coding regions or flanking intron/exon junctions. One A to T substitution was detected in the 3'UTR region of GSTTLp28. This substitution changed the consensus polyadenylation signal from AATAAA to AATAAT. Subsequent analysis of this variant determined that this A to T substitution occurs at polymorphic levels in the population, and is therefore not a likely cause of disease. Additional research is currently underway to characterize and test additional candidate genes for CDB2.

**The elongation of very long chain fatty acids (ELOVL4) gene is not associated with sporadic cases of Stargardt Disease.** *D. DUCROQ<sup>1</sup>, J.-M. ROZET<sup>1</sup>, E. SOUIED<sup>1</sup>, S. GERBER<sup>1</sup>, I. PERRAULT<sup>1</sup>, S. HANEIN<sup>1</sup>, F. BARBET<sup>1</sup>, I. GHAZI<sup>2</sup>, J.-L. DUFIER<sup>2</sup>, A. MUNNICH<sup>1</sup>, K. ZHANG<sup>3</sup>, J. KAPLAN<sup>1</sup>.* 1) Laboratoire de Recherches sur les Handicaps Genetiques de l'Enfant, INSERM U393, Hopital des Enfants Malades, Paris Cedex 15, France; 2) Service d'Ophthalmologie, Hopital Necker, Paris, France; 3) Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, OH 44195.

Stargardt disease has been originally described as an autosomal recessive macular degeneration. It is characterized by a broad clinical heterogeneity contrasting with a surprising genetic homogeneity (a single gene, ABCA4, on 1p22) with clear-cut genotype-phenotype correlations. Nevertheless, some cases of patients have been reported to be affected with a Stargardt-like phenotype inherited as an autosomal dominant trait. One locus has been previously mapped to chromosome 13q34 and recently identified: the elongation of very long chain fatty acids gene (ELOVL4). On the other hand, 80% of patients affected with Stargardt disease are sporadic cases in our series (151 / 188). Surprisingly, in about 50% of these patients, the ABCA4 screening failed to identify any mutation. Therefore we screened the ELOVL4 gene in these sporadic cases of Stargardt. The six exons of the ELOVL4 gene and their intronic flanking regions were analyzed in 77 sporadic cases of STGD by single strand confirmation polymorphism (SSCP) and automatic direct sequencing using specific primers. Only two infrequent intronic polymorphisms were found in two unrelated patients (IVS3-18 C/T, and IVS5+45 delT), respectively. No mutation was found in any of the 6 exons for all patients, excluding ELOVL4 as STGD-causing gene in our series.



**Transcript map of the critical region for keratolytic winter erythema (KWE) on chromosome 8p22-p23 between D8S550 and D8S1759.** S. Appel<sup>1</sup>, K. Reichwald<sup>2</sup>, A. Bergheim<sup>3</sup>, M. Filter<sup>4</sup>, E. Ogilvie<sup>3</sup>, S. Arndt<sup>3</sup>, W. Zimmermann<sup>2</sup>, A. Simmons<sup>5</sup>, M. Lovett<sup>5</sup>, W. Hide<sup>6</sup>, A. Reis<sup>7</sup>, A. Rosenthal<sup>2</sup>, M. Ramsay<sup>3</sup>, H.C. Hennies<sup>1</sup>. 1) Molecular Genetics and Gene Mapping Center, Max Delbrück Center Berlin, Germany; 2) Genome Sequencing Center, Institute of Molecular Biotechnology, Jena, Germany; 3) Human Genetics, The South African Institute for Medical Research, Johannesburg, South Africa; 4) Bioinformatics, Max Delbrück Center Berlin, Germany; 5) Human Genetics, Washington University, St. Louis, USA; 6) South African National Bioinformatics Institute, University of the Western Cape, Bellville, South Africa; 7) Human Genetics, Friedrich Alexander University, Erlangen, Germany.

Keratolytic winter erythema (KWE) is an autosomal dominant skin disorder characterized by erythema, hyperkeratosis, and peeling of the skin of the palms and soles especially during winter. The KWE locus has been mapped to human chromosome 8p22-p23. This region has also been associated with frequent loss of heterozygosity (LOH) in different types of cancer. To identify positional candidate genes for KWE, a BAC contig between D8S550 and D8S1695 was constructed and sequenced. It was extended to D8S1759 by a partially sequenced BAC clone identified by database searches. In the 634,404 bp contig 13 new polymorphic microsatellite loci and 46 single nucleotide and insertion/deletion polymorphisms were identified. Twelve transcripts were found between D8S550 and D8S1759 by exon trapping, cDNA selection, and sequence analyses. They were localized on the genomic sequence, their exon/intron structure was determined, and their expression analyzed. Only one of the transcripts corresponds to a known gene, encoding B-lymphocyte specific tyrosine kinase, *BLK*. A putative novel myotubularin-related protein gene (*MTMR8*), a potential human homologue of the mouse *Amac1* gene, and two transcripts showing similarities to the mouse L-threonine 3-dehydrogenase gene and the human *SEC* oncogene, respectively, were found. The remaining seven transcripts did not show significant similarities to known genes. This identification of novel genes and polymorphisms in the region will facilitate the further analysis of disease loci that map to chromosome 8p22-p23.

**Mutation screening of SF2 and HPRP3P in patients with autosomal dominant Retinitis pigmentosa. C.F.**

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Autosomal dominant Retinitis pigmentosa (adRP) is an inherited progressive retinal degeneration characterised by night blindness and constricted visual fields. adRP primarily affects the rod photoreceptor cell function and the pigment epithelial layer of the retina. adRP is genetically heterogeneous and mutations have been found in two known genes, rhodopsin and peripherin/Rds. Genetic linkage studies have identified several loci for adRP on chromosomes 1q, 7p, 7q, 8q, 17p, 17q and 19q. The basis of our study is screening of candidate genes for adRP on chromosome 1q and 17q. Two candidate genes were investigated the essential pre-mRNA splicing factor (SF2) which influences 5splice site selection and U4/U6 associated splicing factor (HPRP3P). The preliminary linkage analysis on a large Dutch and South African family has localized the region of RP17 in an interval of 7.7 cM on 17q22. The critical interval for RP18 has been narrowed down to 2 cM on 1q21 in a large Danish and English family. SF2 and HPRP3 were selected as appropriate candidate genes for adRP on the bases of their function, expression pattern and gene localization. Two affected and unaffected members of RP17 and RP18 adRP families were subjected to mutation screening by DHPLC system. Four exonic SF2 and fifteen exonic HPRP3P were screened by automatic direct sequencing. RT-PCR was done to show the expression pattern of both genes. GeneBridge4 Radiation Hybrid Mapping Panel was used to find out the approximate localization of SF2 and HPRP3P related to markers with the highest lod score. Both SF2 and HPRP3P are expressed in retina and have wide expression profile. To date one polymorphism each has been identified in both SF2 and HPRP3P. Mutation screening has yet not revealed any mutation in the coding sequence of SF2 and HPRP3P but 5promotor and 3 UTR still remain to be investigated.

**Mutation screening of caveolin-3 and myotilin in autosomal dominant Limb Girdle Muscular Dystrophy and other dominant myopathies.** *R.D. Dancel<sup>1</sup>, J.M. Stajich<sup>1</sup>, J.M. Vance<sup>1</sup>, M.A. Pricak-Vance<sup>1</sup>, P.C. Gaskell<sup>1</sup>, J.M. Gilchrist<sup>2</sup>, R.W. Tim<sup>3</sup>, M.C. Speer<sup>1</sup>, M.A. Hauser<sup>1</sup>.* 1) Section of Medical Genetics, Department of Medicine, Duke University Medical Center, Durham, NC; 2) Rhode Island Hospital, Providence, RI; 3) Raleigh Neurology Associates, Raleigh, NC.

Limb-girdle muscular dystrophy (LGMD) is a genetically heterogeneous condition with both autosomal dominant and autosomal recessive forms and extensive heterogeneity demonstrated within each of the two forms. In the autosomal dominant form of LGMD, loci have been identified on chromosomes 5 (myotilin), 3 (caveolin-3), 1, and 6. To date, only one family has been identified as having a mutation in myotilin. Thus, we undertook a screen of myotilin and caveolin-3 in 32 autosomal dominant LGMD families. We also investigated patients affected with other autosomal dominant myopathies including individuals affected with non-chromosome 4 linked facioscapulohumeral muscular dystrophy, scapuloperoneal muscular dystrophy, and other non-specific dominant myopathies. Each of the 9 coding exons in the myotilin gene and 2 coding exons in the caveolin-3 gene was PCR amplified from genomic DNA in pools of 5 unrelated individuals. The PCR products were analyzed by denaturing high performance liquid chromatography using the Transgenomic WAVE DHPLC system. Several different temperatures were tested for each PCR amplicon to optimize mutation detection sensitivity. Any pools displaying altered retention time were separated and the constituent samples were reanalyzed individually. All observed changes were confirmed by sequencing both strands using the Beckman CEQ2000 capillary electrophoresis sequencer. A single polymorphism, present in both affected and control individuals, was detected in the caveolin-3 gene. To date, no caveolin-3 or myotilin mutations have been detected, excluding mutations in these genes as a common underlying cause of disease in these families.

**Novel mutations in the GDAP1 gene are responsible for Charcot-Marie-Tooth disease type 4A.** *R.V. Baxter, K. Ben Othmane, J.M. Rochelle, J.E. Stajich, J.E. Stenger, M.A. Pericak-Vance, J.M. Vance.* Center for Human Genetics, Duke University Medical Center, Durham, NC.

The Charcot-Marie-Tooth (CMT) phenotype is the most common inherited peripheral neuropathy. The autosomal recessive demyelinating form of CMT, CMT4, was previously sub-divided based on pathological findings, a division that was upheld by later linkage studies. We have previously shown linkage of CMT4A to chromosome 8q21 with four consanguineous families. The minimal candidate region (MCR) was narrowed using haplotype analysis to approximately 300Kb. Screening of candidate genes lying within the region for expression in peripheral nerve demonstrated one candidate, GDAP1, to be highly expressed in this tissue. GDAP1 has been shown to be highly expressed at the Neuro2a differentiated stage, and is expressed in mouse brain at gradually increasing levels over the course of development, peaking at the adult stage (Liu et al, 1999). After analysis of the sequence data for all six GDAP1 exons, the 92<sup>nd</sup> base pair of Exon 1 showed a homozygous G>A change in affected members of two families. This single base-pair change causes a Trp31STOP mutation, which would effectively truncate the protein. A second mutation in Exon 5, shows a C>G change at the 2<sup>nd</sup> base pair in affected family members, which would cause a Ser194STOP mutation, also cutting short the resultant protein. Mutation studies are underway for the fourth family. How the lack of GDAP1 leads to CMT4A remains to be understood, but further study should continue to provide insight into the normal mechanisms of peripheral nerve biology.

**Autoimmune Lymphoproliferative Syndrome (ALPS) Due to Fas-ligand Mutations.** *L. Bi<sup>1</sup>, L. Zheng<sup>1</sup>, J.K. Dale<sup>1</sup>, T.P. Atkinson<sup>3</sup>, J.M. Puck<sup>2</sup>, M.J. Lenardo<sup>1</sup>, S.E. Straus<sup>1</sup>.* 1) LCI & LI/NIAID/NIH, Bethesda, MD; 2) Genet Mol Biol Branch/NHGRI/NIH, Bethesda, MD; 3) Dept Peds/UAB, Birmingham, AL.

ALPS is a disorder of lymphocyte homeostasis due to mutations in gene products that regulate apoptosis. ALPS is classified as Type Ia when there are mutations in APT1 encoding Fas (TNFRSF6); Type II ALPS involves mutations in Caspase-10; in Type III no genetic defects have been found. ALPS Type Ib, with mutations in Fas-ligand (FasL, TNFSF6), has been postulated to exist. We sequenced the coding region for FasL in 32 patients with features suggestive of ALPS and normal Fas. Unique single nucleotide mutations were found in each of 2 unrelated patients but not in 100 chromosomes of normals. The first patient is a 10 yo boy with typical features of ALPS: generalized adenopathy and splenomegaly, idiopathic thrombocytopenic purpura (ITP) and elevated CD4<sup>+</sup>CD8<sup>-</sup> T cells. A heterozygous A530G mutation of FasL cDNA leads to a change of Arg to Gly at peptide site 156, predicted by molecular modeling of a soluble Fas ligand fragment to be part of an external loop of the protein. Cytotoxicity of his PBMCs for Fas positive (Jurkat) cells was reduced compared with PBMCs from normals. The second patient is an 8 yo girl with a history of ITP but no adenopathy or splenomegaly. Sequencing of her FasL showed a heterozygous A320G mutation of cDNA leading a conservative change of Met to Val at peptide site 86 in the predicted transmembrane domain of FasL. To correlate these two mutations with our clinical findings, cDNAs encoding wild type (WT) FasL, both patient variants, and the humanized mouse gld FasL mutant, were cloned into an expression vector, transfected into 293-T cells, and used to kill Jurkat cells. The cells expressing WT and the girls FasL killed Jurkat cells efficiently; gld expressing cells had very low kill activity and cells expressing the boys FasL showed reduced kill activity. Dominant interference studies involving co-transfection of mutant and WT FasL are in progress to elucidate the mechanism of action of these mutations. The cumulative data indicate that only the boy has ALPS Type Ib, and he is the first patient reported to have this diagnosis.

**Dominant vitreoretinal degeneration without systemic manifestations: Stop codon mutation in exon 2 of the COL2A1 gene in a new large family.** A.O. Edwards<sup>1</sup>, R.L. Ritter III<sup>1</sup>, L.A. Donoso<sup>2</sup>. 1) Dept Ophthalmology, Univ Texas SW Medical Ctr, Dallas, TX; 2) Wills Eye Hospital, Philadelphia, PA.

**Objective:** To identify the gene responsible for an autosomal dominant vitreoretinal degeneration occurring in a large family without associated systemic manifestations.

**Methods:** Participating family members were evaluated clinically over a 30 year period. Genetic linkage, genotyping, mutation screening, and an extensive genealogical investigation were performed.

**Results:** We identified a single large family, over 2,000 total family members, with vitreoretinal degeneration spanning 12 generations. We clinically evaluated 165 study participants (95 clinically affected and 70 unaffected). The earliest clinical findings in affected individuals included an optically empty vitreous and posterior perivascular cystic degeneration resembling radial lattice. Later findings included vitreous band formation, multiple retinal tears and detachments predominately along the posterior and peripheral vessels. A cytosine to adenosine (C/A) transition was identified at position 4363 within exon 2 of the *COL2A1* gene, leading to the creation of a stop codon at position 86 (Cys86Stop).

**Conclusions:** A large family with vitreoretinal degeneration without significant associated systemic findings was described. The disease causing mutation was identified as a novel C/A transition in exon 2 creating a stop codon in the *COL2A1* gene. Clinical relevance: This highly specific genotype-phenotype relationship illustrates the importance of vitreoretinal examination in identifying the affected offspring of patients with this disorder. The identification of the disease causing mutation in this family allows for presymptomatic genetic testing to identify family members needing early examination and consideration for prophylactic therapy.

**A mutation in the SLC11A3 gene is associated with autosomal dominant hemochromatosis.** *O.T. Njajou, N. Vaessen, M. Joosse, B. Berghuis, J. van Dongen, M. Breuning, P.L. Snijders, W.P. Rutten, L. Sandkuijl, B.A. Oostra, C.M. van Duijn, P. Heutink.* Genetic-Epidemiology Unit: Departments of Epidemiology & Biostatistics, and Clinical genetics Erasmus University Medical Center, Rotterdam, The Netherlands.

Hereditary Hemochromatosis (HH) is a very common iron disorder in caucasians usually inherited as a recessive trait. The disease is characterized by increased absorption and progressive storage of iron in body tissues resulting in multi-organ damage and widespread pathology including diabetes, cardio-vascular diseases, stroke and cancer. Several genes involved in iron metabolism have been implicated in the pathology of hemochromatosis. Mutations in the HLA-H/HFE gene on chromosome 6p, the transferrin receptor gene (TFR-2) on chromosome 7q have been identified and juvenile hemochromatosis maps to chromosome 1q. However not all HH patients carry these mutations. Also, families with dominant or pseudo-dominant mode of inheritance have been reported. This suggests that other genes are involved in the pathology of hemochromatosis. A very large Dutch family with hemochromatosis has been described in which the disease segregates as a dominant trait that did not map to any known locus. We performed a systematic genome scan for linkage in this family and mapped the disease gene to chromosome 2q. In the critical region, the Solute Carrier family 11 member A3 (SLC11A3) gene also called ferroportin1 has been localized. Subsequent mutation analysis of SLC11A3 identified a single base change in all patients but not in healthy individuals from the family or the general population. This A to C transversion at position 734 (A734C) located in a transmembrane domain of the protein leads to a substitution of asparagine by histidine (N144H) resulting in change of polarity and is predicted to result in excessive SLC11A3 mediated iron transport. We describe for the first time an heterozygous mutation that can explain autosomal dominant hemochromatosis.

**Multiple cutaneous leiomyoma and uterine fibroids: Confirmation and refinement of the *MCUL1* locus. A.**

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Uterine leiomyomas are common benign tumors of smooth muscle origin. Although it is a leading cause of hysterectomy, little is known about the molecular mechanisms underlying these tumors. Genetic predisposition, steroid hormone concentrations, and environmental factors have been suggested to play a role in the pathogenesis of these tumors (Stewart, 2001). The association of uterine leiomyomas (myomas or fibroids) with cutaneous leiomyomas in an autosomal dominant syndrome called multiple cutaneous leiomyoma (MCL [MIM 150800]) facilitates the study of the genetic causes underlying these tumors.

Very recently, the locus responsible for MCL has been mapped to a ~14 cM region on chromosome 1q42.3-43 (Alam et al, 2001). Moreover, the same locus has been identified in a separate study as the susceptibility locus for a hereditary cancer syndrome including renal cell cancer, as well as uterine and cutaneous leiomyoma (Launonen et al, 2001). Here, we report the clinical and genetic findings in an extended Israeli pedigree with MCL. We describe the clinical features of MCL in a new large family, with emphasis on the particular timing and specific pattern in the appearance of the skin lesions. The genetic analysis of the family has allowed us to narrow the location of the *MCUL1* locus to a region of 4-7 cM on chromosome 1.



**The mutations in the untranslated *RMRP* gene that result in cartilage-hair hypoplasia, a pleiotropic human disease.** *I.I. Kaitila*<sup>1, 2, 3</sup>, *M. Ridanpää*<sup>1, 2, 3</sup>. 1) Clinical Genetics Unit, Helsinki Univ Central Hosp,; 2) Department of Medical Genetics, Univ Helsinki,; 3) Folkhälsan Institute of Genetics, Helsinki, Finland.

We have recently demonstrated that mutations in *RMRP* gene are the cause of cartilage-hair hypoplasia (CHH), an autosomal recessive multiorgan condition (Ridanpää et al., Cell, 2001, 204:197 - ). The primary transcript of the gene consists of 267 nucleotides, only, and it encodes the RNA component of an endoribonuclease RNase MRP that acts in cleavage of RNA in mitochondrial DNA synthesis and nucleolar cleaving of pre-rRNA. Other functions probably exist. The clinical manifestations of CHH consist of short stature due to metaphyseal chondrodysplasia, hair hypoplasia, defective erythropoiesis presenting with refractive anemia, defective cell-mediated, and often, humoral immunity, defective spermatogenesis, variable Hirschsprung disease, and risk of malignancies. CHH is common among the Amish in the USA and the Finns, and rare in other populations. In order to understand the pathogenetic mechanisms in CHH we screened 91 Finnish and 36 non-Finnish CHH patients for *RMRP* mutations. We found that 84 per cent of the Finnish patients were homozygous for nt70A->G substitution, and 13 per cent were compound heterozygotes with nt70A->G/nt262G->T. The nt70A->G was also observed in 25 chromosomes out of the 36 patients from other populations. In particular, nt 70A->G homozygotes were found in Canada, England, Turkey, USA, and the Netherlands whereas compound heterozygotes with variable allelic mutations in Australia, Brazil, Canada, England, Germany, and USA. Thus, nt70A->G is probably an ancient mutation. Other types of mutations were substitutions, insertions, duplications and deletions located either upstream of the coding sequence or in the transcribed region of the gene. The allele carrying an insertion or deletion in the region upstream of the coding sequence probably does not express. - The mutation screening can be offered as a diagnostic test in a suspected case of CHH. *RMRP* is the first untranslated gene the product of which has functions in the nucleolar and mitochondrial RNA biochemistry.

**Transcriptome analysis of the *rd1* mouse.** *T. Leveillard*<sup>1</sup>, *C. Lavedan*<sup>2</sup>, *S. Mohand-Säid*<sup>1</sup>, *M. Dressman*<sup>2</sup>, *A. Dölemeyer*<sup>3</sup>, *G. Lambrou*<sup>3</sup>, *J. Sahel*<sup>1</sup>. 1) INSERM/ULP/HUS EMI 99-18, Strasbourg France; 2) Pharmacogenetics, Novartis, Gaithersburg, MD; 3) Novartis Ophthalmics, Basel, Switzerland.

In the *rd1* mouse, an animal model of Rod-Cone dystrophy, the loss of rod photoreceptors results from a mutation in the rod b-Phosphodiesterase gene. This leads to cone degeneration by deprivation of Rod-dependant Cone Surviving Factors. Reintroducing these factors to prevent vision loss is a valuable therapeutic approach of Retinitis Pigmentosa (RP). To identify genes whose expression is lost, we have performed gene expression analysis of neural retina following rod degeneration using Affymetrix microarray technology. Among the ~9,800 genes studied, 1,229 had a difference in expression of at least three fold between *rd1* and wild type samples. The expression of 640 genes was reduced in the absence of rods and 589 genes had an elevated expression in the *rd1* mouse neural retina at five weeks compared to wild type. The apparent lower expression of certain genes in *rd1* could be a direct consequence of the loss of rod cells or indirectly of other cells dependent on rods. The kinetics of expression of these genes during rod degeneration was studied using RT PCR. Their human orthologues are candidate genes that may be mutated in RP. Genes with higher expression in *rd1* are induced by the degeneration of rod cells; they are upregulated by the cones of the outer retina and/or other cells of the inner retina. We classified the 1,229 genes with differential expression according to their known function. The percentage of Transcription Factor and Signal Transduction genes did not significantly vary between the *rd1* and wild type samples, reflecting the complexity of signaling involved in rod maintenance or in the response to rod degeneration. The majority of up-regulated genes were classified as Chaperone, Apoptosis, Cell Adhesion and Redox control genes and might reflect stress signaling in response to rod degeneration. Gene expression profile analysis of rod degeneration in a mouse model of RP provides a new way to identify genes and pathways implicated in retinal function, and neuro-protective molecules with therapeutic potential.éé.

**The Polynucleotide Kinase 3-Phosphatase Gene is not Responsible for Autosomal Recessive Charcot-Marie-Tooth Disease type 2 in Chromosome 19q13.3.** A. Leal<sup>1,2</sup>, B. Morera<sup>3</sup>, G. Del Valle<sup>4</sup>, D. Heuss<sup>5</sup>, C. Kayser<sup>5</sup>, M. Berghoff<sup>5</sup>, B. Neundoerfer<sup>5</sup>, R. Barrantes<sup>2</sup>, A. Reis<sup>1</sup>, B. Rautenstrauss<sup>1</sup>. 1) Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen, Germany; 2) Institute of Health Research (INISA) and School of Biology, University of Costa Rica, San Jose, Costa Rica; 3) Unitat de Biologia Evolutiva, Universitat Pompeu Fabra, Barcelona, Spain; 4) Department of Neurology, San Juan de Dios Hospital, San Jose, Costa Rica; 5) Department of Neurology, University of Erlangen-Nuremberg, Erlangen, Germany.

Charcot-Marie-Tooth disease (CMT), a group of hereditary motor-sensory neuropathies, is a clinically and genetically heterogeneous disorder of the peripheral nervous system. Recently, we found linkage in a Costa Rican family with autosomal recessive CMT type 2 (ARCMT2B) in chromosome 19q13.3. The critical interval was defined between markers D19S902 and D19S907 (5.5 cM). Clinically, the age at onset of chronic symmetric sensory-motor polyneuropathy was 28 to 42 years (mean 33,8) in this family, and the electrophysiologic data clearly reflect an axonal degenerative process. After localizing the gene, contigs of the region were reconstructed *in silico*, and using several programs (i.e. NIX program) 72 unigene-clusters were identified in the critical region. The genomic sequence of the interval was compared automatically (HUSAR package, DZKF) against 15,000 sequences from five peripheral nerve cDNA libraries (CGAP, NCI). The Polynucleotide kinase 3-phosphatase gene (*PNKP*) was found being present in the critical interval and expressed in peripheral nerve. PNKP is a DNA-specific kinase, and it is predictive of a function in DNA repair. Intronic primers for testing this gene were designed based in the exon-intron structure, obtained comparing the cDNA sequence (AF120499) against the genome sequence (AC021163), and was checked for any alternative splicing reported until know (Alternative Splice Finder program). Finally, this positional and expressional candidate gene was analyzed by direct sequencing, however, no mutation was found in patients with ARCM2B in the Costa Rican family.

**Analysis of candidate genes for Hereditary Neuralgic Amyotrophy (HNA).** *J.R. Meuleman, G.D.J. Watts, K.C. O'Briant, P.F. Chance.* Department of Pediatrics, University of Washington, Box356320, Seattle, WA 98195.

Hereditary Neuralgic Amyotrophy (HNA) is an autosomal dominant disorder characterized by sudden, painful episodes of brachial plexus neuropathy. Linkage analysis defined a locus on chromosome 17q25, which was recently narrowed down to a 1 Mb interval. Furthermore, 4 positional candidate genes had been excluded recently. Here we present the analysis of 6 known positional candidate genes (the *ET* transcripts, *KIAA1067*, *KIAA0585*, *LAK4P*, *PRPSAP1* and *SYNGR2*) and one novel positional candidate gene homologous to the *MGAT5* gene. The genes were studied in affected and control individuals from 6 — 9 HNA families, which were previously linked to the 17q25 HNA locus. We performed mutation analysis by sequencing the coding regions at cDNA or genomic DNA. No nucleotide changes were detected in the *ET* transcripts, *KIAA1067*, *KIAA0585*, *LAK4P*, *PRPSAP1* and *SYNGR2*. The last gene we analyzed was deduced from the hypothetical protein DKFZp761J107 using RT—PCR, Genscan predictions and the apparent homology with the *MGAT5* gene. We determined the genomic structure of 18 exons by alignment with the genomic sequences of RP11—87G24. Mutation analysis showed 5 silent base changes (1203G>C, 1338C>T, 1476C>T, 2022C>T and 2316C>T) and 2 base changes causing an amino acid substitution (208G>A and 661G>A leading to I70V and G221R respectively). However, none of these coding SNPs could be linked with the HNA phenotype since they were present in both affected and unaffected, married-in individuals. Furthermore, the exclusion was confirmed by the recent refinement of the HNA linkage interval. Hereby we excluded all known positional candidate genes.

**A missense mutation of human Gephyrin (*GPHN*) is associated with Hyperekplexia and transcript isoform analysis re-defines the genomic structure of *GPHN*.** M.I. Rees<sup>1</sup>, K. Baer<sup>1</sup>, H. Ward<sup>1</sup>, S.L. Coleman<sup>2</sup>, I.L. Evans<sup>3</sup>, J. Miller<sup>1</sup>, H. Waldvogel<sup>4</sup>, R.L.M. Faulk<sup>4</sup>, M.J. Owen<sup>2</sup>, R.G. Snell<sup>1</sup>. 1) Department of Molecular Medicine, University of Auckland Medical School, Auckland, New Zealand; 2) Department of Psychological Medicine, University of Wales College of Medicine, Cardiff, U.K; 3) University of Birmingham Medical School, Birmingham, U.K; 4) Department of Anatomy, University of Auckland Medical School, Auckland, New Zealand.

Gephyrin (*GPHN*) is an organisational protein that clusters and localises the inhibitory glycine (GlyR) and GABAA receptors to the microtubular matrix of the neuronal postsynaptic membrane. Mice that are deficient in gephyrin develop a hereditary molybdenum cofactor deficiency and a neurological phenotype that mimics startle disease (hyperekplexia). We have previously confirmed genetic heterogeneity for hyperekplexia by demonstrating mutations in the  $\alpha 1$ -subunit (*GLRA1*) and  $\beta$ -subunit (*GLRB*) of the GlyRs. However, the exclusion of *GLRA1* and *GLRB* mutations in a proportion of our sporadic sample of patients, indicates the presence of additional genetic determinants and candidate gene analysis of *GPHN* was undertaken. Following the *in silico* determination of the genomic structure of *GPHN* from chromosome 14 BACS, mutation analysis of all 27 *GPHN* exons in hyperekplexia patients revealed several polymorphisms, and most significantly, a missense mutation (A28T) in exon 1 that causes an amino acid substitution (Asn10Tyr). This change disturbs a myristylation consensus region at the N-terminus of *GPHN* and we speculate that this affects binding of gephyrin to the postsynaptic plasma membrane and compromises the gephyrin scaffold. Preliminary data from neuronal and non-neuronal human RNA isoforms confirm the presence of 27 *GPHN* exons (5 alternatively spliced), whereas the existence of 29 exons (7 alternatively spliced) in rat tissues raises the possibility of additional uncharacterised isoforms. This work will provide a basis for mapping gephyrin isoform distribution in human brain.

**Mutation and deletion analysis of the Van der Woude syndrome locus at chromosome 1q32-q41.** *B.C. Schutte<sup>1,2</sup>, Y. Watanabe<sup>1</sup>, B.C. Bjork<sup>2</sup>, E.L. Howard<sup>3</sup>, S.A. Hoper<sup>1</sup>, M.I. Malik<sup>1</sup>, M.M. Fang<sup>1</sup>, P.I. Hemerson<sup>1</sup>, M.J. Dixon<sup>3</sup>, J.C. Murray<sup>1,2</sup>.* 1) Dept Pediatrics, Univ Iowa, Iowa City, IA; 2) Genetics PhD Program, Univ Iowa, Iowa City, IA; 3) Univ Manchester, Manchester, UK.

Van der Woude syndrome (VWS) is an autosomal dominant orofacial clefting disorder (OMIM #119300). Characteristics of VWS include lower lip pits, cleft lip with and without cleft palate, isolated cleft palate, hypodontia and normal intelligence. The VWS locus maps to a 1.6 cM region between markers D1S491 and D1S205 on chromosome 1q32-q41. The finished sequence for this region is available, and we have identified 41 genes within and adjacent to the 363 kb critical region. Using SSCP, we screened 137 exons that contain an open reading frame for mutations in 90 probands. We identified 25 mutations, but none were etiologic for VWS. Previously, we observed that deletion of the VWS critical region was etiologic in two families. Many deletions would not be detected by SSCP analysis, so we also performed an allele loss assay to screen for new VWS-causing deletions. We genotyped 30 VWS triads with 22 single nucleotide polymorphisms (SNPs) from the SNP consortium (<http://snp.cshl.org>). The average heterozygosity for these SNPs was 40% and 43% in CEPH control and VWS populations, respectively. We did not observe an allele loss with any of the SNPs in any of the 30 families tested. However, we observed a large region of monozygosity in a single pedigree (family 1470) that is consistent with the presence of a deletion. This region contains seven markers, including the STRP D1S3753 which is located in the middle of the monozygous region and has 85% heterozygosity. Based on the combined heterozygosities of the seven markers, there is a 1.7% probability that the observed monozygosity is a random event. Southern analysis was performed using a probe inside the monozygous region (5 kb proximal to D1S3753) and a control probe from a different chromosome. No differences were seen between the proband and control samples. We conclude that the region proximal to D1S3753 is not deleted in family 1470, and more generally, that deletions larger than 20 kb do not significantly contribute to VWS etiology.

**Candidate gene screening of periaxin and its interactors in Charcot-Marie-Tooth neuropathy.** *H. Takashima*<sup>1</sup>, *C.F. Boerkoel*<sup>1</sup>, *D.L. Sherman*<sup>3</sup>, *P.J. Brophy*<sup>3</sup>, *J.R. Lupski*<sup>1,2</sup>. 1) Mole Human Gen/TX Med Ctr, Baylor Col Medicine, Houston, TX; 2) Department of Pediatrics, Texas Childrens Hospital, Houston, TX; 3) Department of Preclinical Veterinary Sciences, University of Edinburgh, Edinburgh, UK.

Charcot-Marie-Tooth neuropathy (CMT), one of the most common inherited neurologic diseases, is clinically and genetically heterogeneous. At least 15 genetic loci and 9 genes have been associated with CMT and related neuropathies; however, we have ascertained 168 CMT families without an identified gene mutation. Therefore this observation suggests that mutations in other genes must also cause CMT. We hypothesized that mutations in Periaxin (PRX), or proteins interacting with Periaxin, might cause demyelinating neuropathy because PRX<sup>-/-</sup> mice develop a severe demyelinating neuropathy. We performed denaturing high-performance liquid chromatography (DHPLC) and DNA sequencing analysis to screen for mutations of these genes in 168 families without mutations involving PMP22, MPZ, GJB1, EGR2 and MTMR2. We identified three Dejerine-Sottas neuropathy (DSN) patients with recessive PRX mutations; two had compound heterozygous nonsense and frameshift mutations and one had a homozygous frameshift mutation. Sequence alterations of proteins interacting with Periaxin have also been identified and the association of these alterations with disease is being evaluated.

**LAMA2 mutation thought to be molecular basis of congenital muscular dystrophy in the merosin-deficient *dy2j* mouse is found to be splicing variant present in normal mice.** *D.M. Pillers, J. Pang.* Dept Pediatrics and Molecular & Medical Genetics, Oregon Health Sci Univ, Portland, OR.

Laminin is a trimeric extracellular glycoprotein that is a component of the extracellular matrix. It plays an important role in cell adhesion and migration, in addition to signaling through transmembrane molecules such as integrin and dystroglycan. Laminin-2, also known as merosin, is critical in skeletal muscle development and function. Mutations in laminin-a2 are associated with human congenital muscular dystrophy (CMD) and with muscular dystrophy in the mouse models *dy* and *dy2j*. A G to A splice donor mutation at exon 2 in *dy2j* was defined by Xu et al. (1994) as the site of the mutation resulting in the muscular dystrophy phenotype based upon the creation of a group of aberrant laminin-a2 transcripts. In one, exon 2 is skipped but the reading frame is retained. In others, a stop codon is predicted to result in a truncated protein. We performed RT-PCR analysis of the alternatively spliced products for laminin-a2 and found that the spliced-in fragment described by Xu et al. is present in both normal C57Bl/6J mice and the muscular dystrophy mouse model *dy2j* suggesting that the mutation may actually be a polymorphism. Furthermore, we found that the truncated products predicted by Xu et al. could be translated into near full-length proteins, lacking only domain VI of the laminin-a2 subunit which contains the binding sites for heparin and cell surface receptors. We also found that the skipping pattern of exon 2 resulted in skipping the entire exon 2 while retaining the reading frame, as opposed to skipping part of exon 2 and the entire exon 3 as suggested by Xu et al. Thus, we propose that the splicing-in variant is expressed in normal mouse and likely has a specific function. In addition, we propose that the alternative splice pattern occurs independent of the G to A polymorphism and is the result of a novel regulatory mechanism.



**SOD -A multigenic disorder?** *K.S. Woods, D. McNay, J.P.G. Turton, M.T. Dattani.* Biochem/Endoc/Metabolism Unit, Institute of Child Health, London, England.

Development of the anterior pituitary gland is a multigenic process regulated by complex signalling pathways which determine expression and interaction of a number of transcriptional factors. Fate map analysis reveals that the anterior neural ridge will give rise to the anterior pituitary and olfactory lobe whereas adjacent regions of the neural plate give rise to the hypothalamus, posterior pituitary, optic vesicles and ventral forebrain. We have previously described the 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 mutations in this gene account for only a small number of cases of SOD (8/570). We have now screened a large cohort (n=205) of SOD patients with variable phenotypes for a number of candidate genes implicated in forebrain and pituitary development

The genes selected for this on going study (*EMX2, PAX6, 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. Whilst to date no definitive mutations have been documented, a number of polymorphisms, both exonic and intronic, have been identified.

Two intragenic polymorphisms have previously been described in *SIX6*<sup>(2)</sup>, one of which is within the homeobox of this gene. This polymorphism appeared to be present at a high frequency within our cohort of patients and further characterisation within a control population using pyrosequencing technology confirmed a similar prevalence.

Although these polymorphisms are present in control populations, they may well lead to a change in protein function e.g. an N125S polymorphism within *HESX1* (MT Dattani;personal communication). Interactions with other proteins and/or the environment may therefore be crucial factors in the manifestation of a phenotype.

<sup>1</sup>(Dattani *et al.* 1998) <sup>2</sup>(Gallardo *et al.* 1999).

**Towards the identification of at least 12 genes in Leber congenital amaurosis.** *I. PERRAULT<sup>1</sup>, S. GERBER<sup>1</sup>, S. HANEIN<sup>1</sup>, J.-M. ROZET<sup>1</sup>, D. DUCROQ<sup>1</sup>, F. BARBET<sup>1</sup>, I. GHAZI<sup>2</sup>, J.-L. DUFIER<sup>2</sup>, A. MUNNICH<sup>1</sup>, J. KAPLAN<sup>1</sup>.* 1) Laboratoire de Recherches sur les Handicaps Genetiques de l'Enfant, INSERM U393, Hopital des Enfants Malades, PARIS Cedex 15, FRANCE; 2) Service d'Ophtalmologie, Hopital Necker, PARIS, FRANCE.

Leber congenital amaurosis (LCA) is an autosomal recessive condition responsible for congenital blindness or greatly impaired vision since the first months of life. A subtle clinical variability has long been reported but largely ignored. Conversely, the genetic heterogeneity of this condition no more needs to be demonstrated, as since 1996, six different genes have been identified. All together, these six genes account for about 45% of all cases. These genes are unequally responsible for the disease in our series of 145 LCA patients: the retinal-specific guanylate cyclase (retGC1) gene in 22%, the retinal pigment epithelium 65 KDa (RPE65) gene in 5.5 %, the cone-rod homeobox containing gene (CRX) in 1.4%, the Aryl Hydrocarbon-interacting protein-like (AIPL1) gene in 5.5%, the retinitis pigmentosa GTPase regulator-interacting protein (RPGRIP1) gene in 5.5% and the crumb homolog (CRB1) gene in 5%. All six genes are involved in very different physiopathologic pathways. Genotype-phenotype correlations clearly demonstrate that the most severe phenotype is related to retGC1 mutations which constantly lead to a dramatic stationary cone-rod dystrophy since birth. Conversely, the milder phenotype is related to RPE65 mutations which are responsible for a very early but progressive rod-cone dystrophy, consistent with a poor visual fonction in the first years of life. The four other gene mutations account for a profound visual impairment which severity varies between the two extreme ends represented by retGC1 and RPE65, respectively. Genome-wide searches for other LCA genes failed to map another major locus comparable to retGC1. Therefore it is very likely that numerous genes remain to be identified so that to account for the remaining 55% patients. Consequently, we speculate that at least six additional LCA genes should be identified, each of them accounting for a small proportion of patients.

**Dominant X linked RP are constantly accounted for by truncating mutations in the ORF 15 exon of the RPGR gene.** *J.-M. ROZET<sup>1</sup>, I. PERRAULT<sup>1</sup>, N. GIGAREL<sup>1</sup>, E. SOUIED<sup>1</sup>, I. GHAZI<sup>2</sup>, S. GERBER<sup>1</sup>, J.-L. DUFIER<sup>2</sup>, A. MUNNICH<sup>1</sup>, J. KAPLAN<sup>1</sup>.* 1) Unite de Recherches sur les Handicaps Genetiques de l'Enfant, INSERM U393, Hopital des Enfants Malades, Paris, Cedex 15, FRANCE; 2) Service d'Ophtalmologie, Hopital Necker, Paris, FRANCE.

Retinitis pigmentosa (RP) is a group of progressive hereditary disorders of the retina in which various modes of inheritance have been described. In 1997, we reported on X-linked RP in nine families with constant and severe expression in carrier females. However, the phenotype was milder and delayed in females compared to hemizygous males. This form of X-linked RP could be regarded therefore as partially dominant (DXLRP). The disease gene has been mapped to chromosome Xp21 in the genetic interval encompassing the RP3 locus ( $Z_{max} = 13.71$ ). At this time, direct sequence analysis of the retinitis pigmentosa GTPase regulator (RPGR) gene, which accounts for RP3, failed to detect any mutation in our families. Very interestingly, in 2000, an additional RPGR exon (ORF15 exon) has been identified and reported to be a hot spot for mutations in recessive X-linked RP. Subsequently we undertook the screening of this novel exon in the nine original DXLRP families as well as in 11 additional cases. Fifty-five percent of these 20 families were found to harbour a truncating mutation in the ORF15 exon of RPGR gene. Interestingly, in two of these families, the mutation was identified in two asymptomatic potential carriers. This finding confirms the incomplete penetrance of the gene and improves genetic counselling in families affected with DXLRP.

**Identification of Novel Retina and Cochlea Specific Genes in the Critical Region for Usher Syndrome Type III.**

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Usher syndrome type 3 (USH3; MIM 276902) is an autosomal recessive disorder associated with progressive hearing loss, variable vestibular responses and adolescent onset retinitis pigmentosa (RP). A locus for USH3 was originally mapped to a 5 cM region on chromosome 3q25, and has recently been refined to a 250-kb genomic fragment between markers 107G19CA7 and D3S3625 by a group in Finland (Tarja J et al; Genomics 63, 409-416, 2000). Using a positional candidate approach, cDNA library screening, RACE and cloning in silico, we identified two USH3 candidate genes USH3Ca and USH3Cb in the USH3 critical region. Both genes are expressed in retina and inner ear. The size of transcripts of both genes is approximately 10-11 kilobases. USH3Ca mRNA has 3 exons and a large open reading frame (ORF) encoding a predicted protein of 2365 amino acids. This predicted protein has multiple immunoglobulin like domains and high homology to adlican (AAF86402), a novel protein expressed in human arthritic tissues. The USH3Cb mRNA has 39 exons and an ORF of 6438 nucleotides. Predicted USH3Cb protein has high homology to human thyroid hormone receptor-associated protein (NP\_005111) and *Drosophila m.* protein kohtalo (AAG48320). The characterization of USH3 candidate genes provides the basis for mutational screening of USH3 families to identify the gene mutated in Usher Syndrome type III.

**Clinical and genetic heterogeneity in connexin disorders of the skin.** *G. Richard*<sup>1</sup>, *A. Krol*<sup>2</sup>, *F. Rouan*<sup>1</sup>, *N. Brown*<sup>1</sup>, *J.J. DiGiovanna*<sup>3</sup>, *S.J. Bale*<sup>4</sup>, *J. Uitto*<sup>1</sup>. 1) Dermatology & Cutaneous Biol, Thomas Jefferson Univ, Philadelphia, PA; 2) Division of Dermatology and Cutaneous Sciences, University of Alberta, Edmonton, Canada; 3) Department of Dermatology, Brown University, Providence, RI/NCI, NIH; 4) NIAMS, NIH, Bethesda, MD/GeneDx, Rockville, MD.

Dominant mutations in at least 4 connexin genes expressed in ectodermal tissues may impair hearing, epidermal differentiation, or both. Erythrokeratoderma variabilis (EKV) is an inherited disorder of the skin caused by mutations in *GJB3* (Cx31) or, as recently suggested (Macari et al., Am J Hum Genet 2000), in *GJB4* (Cx30.3). In a large series of 23 unrelated EKV families, 12 were found to carry heterozygous missense mutations in *GJB3* including 4 novel mutations (G12S, G45E, S26T and L209F). In addition, 5 EKV families harbored pathogenic mutations in *GJB4* affecting a conserved residue in the N-terminus (G12D) or the transmembrane domains of Cx30.3 (T85P, F137L and F189Y). While these defects in Cx31 and Cx30.3 produce a common EKV phenotype with subtle differences in erythema and hyperkeratosis, dominant mutations in the Cx26 gene have a pleiotropic effect, causing either hearing impairment alone or associated with distinct forms of palmoplantar keratoderma (PPK). We identified a family segregating a novel *GJB2* mutation (N54K) substituting a highly conserved asparagine in the first extracellular loop of Cx26. Besides deafness and PPK, this mutation resulted in development of prominent knuckle pads and leukonychia, a disorder diagnosed as Bart-Pumphrey syndrome. Our observations extend the phenotypic spectrum of Cx26 mutations to include also nail abnormalities. The striking clinical overlap between defects in Cx26, Cx31, Cx30.3 and Cx30 emphasizes the importance of gap junctions for the normal development and function of ectodermal tissues.

**Chromosomal duplication at the *IRIDI* locus on 6p25 associated with wide variability of the glaucoma phenotype.**

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Mutations in *FOXC1*, a forkhead/winged-helix transcription factor located at the *IRIDI* locus on 6p25, cause defects of the anterior chamber of the eye associated with developmental glaucoma. We observed a large French-Canadian family: pedigree BV-001, in which 30 patients showed wide phenotypic variability, ranging from asymptomatic anomalies of the eye to primary open-angle glaucoma (POAG). The disorder was linked to the *IRIDI* locus; however, no mutations were identified in the coding region of *FOXC1*. To characterize the genetic defect, a high resolution physical map was constructed between D6S1600 and D6S344 using 89 BACs/PACs and "in silico" mapping. Our contig spanned 500 kb and encompassed two other candidate genes: *FOXQ1* (previously known as *HFH1*) and *FOXF2*, also harboring forkhead/winged domains. No coding mutations were detected in either genes. Two novel SNPs positioned the disease interval centromeric to *FOXQ1*. Four microsatellites: 668J24-GT, AFMb034ya5, UT7184 (D6S967) and 118B18-CA47, showed a three allele system in branches of the pedigree. Segregation analysis of these markers identified the presence of a small 6p25 duplication in affected persons carrying the disease haplotype. The minimal duplicated region spanned from 668J24-GT to 118B18-CA47, about 12 kb telomeric to *FOXC1*, and encompassed *FOXF2*. Our data suggest that duplication events at 6p25 may be associated with mild ocular abnormalities as well as with wide variability of the glaucoma phenotype, including typical POAG. This duplication event may increase dosage of *FOXC1* and/or *FOXF2* and/or alter the transcriptional activity of these two genes by unrecognized positional effects.

**Mutation analysis of two candidate genes in the paroxysmal dystonic choreoathetosis locus on chromosome 2q.** *D. Tokarz, D. Thomas, S.R. Rainier, J.K. Fink.* Department of Neurology, University of Michigan, Ann Arbor, MI.

Paroxysmal dystonic choreoathetosis (PDC) is a movement disorder characterized by involuntary movements affecting the extremities, trunk and face. Episodes last from minutes to hours and are generally brought on by stress or ingestion of alcohol or caffeine. The disorder was linked to 2q (Fink et al. *Am. J of Hum Genet.* 59:140-145, 1996 and Fouad et al. *Am. J. Hum. Genet.* 59:135-139, 1996) and the locus was reduced to a 2.7 cM locus by the work of a number of laboratories including Hofele et al. *Neurology* 49:1252-1257, 1997, and Jarman et al. *Neurology* 48:A996, 1997. Since many other movement disorders are caused by mutations in ion channel genes, ion channel genes are considered to be primary candidates for PDC. There are two ion channel genes in the reduced PDC locus, AE3 (solute carrier family 4, member 3) and ASIC4 (a putative acid sensing channel). These ion channel genes have already been examined by our laboratory and others. There are no disease specific mutations in the coding regions or splice junctions of these genes. We have examined two other candidate genes in this locus, SLC11A1 and flj13021. SLC11A1 is a proton coupled divalent metal ion transporter, also known as NRAMP1 for its function as a natural resistance-associated macrophage protein. We sequenced the 14 exons of this gene as well as the splice junctions from 3 affected and one unaffected individual from our PDC pedigree. No disease specific mutations or polymorphisms were identified. The second gene flj13021 is 82% homologous to rat phospholipase C delta 4 gene at the nucleotide level and 63% and 51% homologous with rat and bovine phospholipase C, respectively, at the protein level. Since phospholipases are important in intracellular signaling pathways, flj13021 was considered a possible candidate for PDC. Flj13021 has 14 exons which were sequenced along with the splice junctions from 4 affected members of our PDC pedigree. Although several polymorphisms were found, no disease specific mutations were identified. There are a number of other genes in the locus which are important in cell signaling. Mutation analysis of these PDC candidate genes is underway.

**Exclusion of interleukin-17 (cytotoxic T-lymphocyte-associated serine esterase 8) as a candidate for autosomal recessive polycystic kidney disease.** *L.C. Wilkins<sup>1</sup>, W.S. Davidson<sup>1</sup>, P.S. Parfrey<sup>2</sup>, J.S. Green<sup>2</sup>, E. Dicks<sup>2</sup>.* 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Faculty of Medicine, Memorial University of Newfoundland, St. John's, Nfld., Canada.

Autosomal recessive polycystic kidney disease (ARPKD) is one of the most common hereditary diseases in children affecting the kidneys and biliary tract. The locus, PKHD1, has been mapped to chromosome 6p21.1-p12. Using a combination of microsatellite and SNP markers, we have been able to reduce the critical region to approximately 580 Kbp whose sequence has been determined as part of the Human Genome Project (NT\_019428). Only two genes, MCM3 (minichromosome maintenance deficiency) and interleukin-17 (cytotoxic T-lymphocyte-associated serine esterase 8) and a suspected pseudogene, carnitine/acylcarnitine translocase, have been identified in this region. MCM3 has previously been excluded as a candidate for PKHD1. Primers were designed that amplify the exons and exon-intron boundaries of the interleukin-17 gene. We used these to determine the corresponding sequences in a large multiplex Newfoundland family in which there are five affected individuals in two branches, one of which is known to be consanguineous. No variation from the published sequence was detected except for a known SNP in the 3' untranslated region of the gene. Thus, it would appear that interleukin-17 can be excluded as a candidate for PKHD1. We are currently examining the structure of the suspected pseudogene in this family and investigating the possibility that an as yet undetected gene exists in this segment of the genome. (Supported by funds from the Kidney Foundation of Canada and the Canadian Institutes of Health Research.).



**Abnormal Expression of Immune Function Genes in B Cell Lines from ICF Syndrome Patients with Known Mutations in DNA Methyltransferase 3B Alleles.** *M. Ehrlich*<sup>1</sup>, *G. Jiang*<sup>1</sup>, *F. Tsien*<sup>1</sup>, *K. Buchanan*<sup>2</sup>, *W. Vicker*<sup>2</sup>, *D. Misek*<sup>3</sup>, *R. Kuick*<sup>3</sup>, *S. Hanash*<sup>3</sup>. 1) Dept. Human Genetics, SL31, Tulane Medical Sch., New Orleans, LA; 2) Dept. Microbiol. Immunol., Tulane Medical Sch., New Orleans, LA; 3) Dept. Ped. Hematol. Oncol., Univ. Mich., Ann Arbor, MI.

ICF (immunodeficiency, centromeric region instability, facial anomalies) is a recessive disease caused by mutations in the DNA methyltransferase 3B gene (*DNMT3B*). Patients invariably have immunodeficiency, chromosome 1 and 16 pericentromeric anomalies in mitogen-stimulated lymphocytes, and a small decrease in genomic 5-methylcytosine levels. Serum immunoglobulin (Ig) levels and numbers of B cells and T cells in blood samples are highly variable among ICF patients but the typical finding is low serum Ig coupled with normal B and T cell numbers in peripheral blood. Polyclonal B lymphoblastoid cell lines (LCLs) from five ICF patients with diverse mutations in their *DNMT3B* alleles were compared with analogous normal LCLs by microarray analysis of expression of about 6000 genes and by flow cytometry for detection of surface immunoglobulins. Four of the five ICF LCLs were highly positive for cell surface IgM (sIgM<sup>+</sup>) and moderately sIgD<sup>+</sup>, and all were sIgG<sup>-</sup> and sIgA<sup>-</sup>. In contrast, most of the normal LCLs had a large fraction of sIgG<sup>+</sup> or sIgA<sup>+</sup> cells and a very small fraction of sIgD<sup>+</sup> or sIgM<sup>+</sup> cells. Furthermore, all the ICF LCLs had high levels of IgM and IgD heavy chain RNAs but very low levels of IgG3, IgA1, and IgA2 heavy chain RNAs compared to the control LCLs. Among the genes that were dysregulated specifically in the ICF LCLs, as revealed by microarray analysis, were nine whose functional properties can help explain the particular patterns of immunodeficiency seen in ICF patients. These genes are involved in lymphocyte activation, migration, or survival and are being analyzed to determine how *DNMT3B* mutations lead to the increased RNA levels for some of them and decreased levels for others. (Supported by PHS Gt. CA81506.).

***p63* mutations are not a major cause of non-syndromic SHFM.** X.J. de Mollerat<sup>1,2</sup>, D. Everman<sup>1</sup>, K. Clarkson<sup>1</sup>, C. Rogers<sup>1</sup>, A. Aylsworth<sup>3</sup>, J. Graham<sup>4</sup>, R.E. Stevenson<sup>1</sup>, C.E. Schwartz<sup>1,2</sup>. 1) Greenwood Genetic Center, South Carolina; 2) Department of Genetics and Biochemistry, Clemson University, South Carolina; 3) Departments of Pediatrics and Genetics, University of North Carolina, Chapel Hill; 4) Cedars Sinai Medical Center, UCLA, California.

EEC syndrome is an autosomal dominant disorder characterized by ectodermal dysplasia, ectrodactyly and cleft lip/palate. Recently, missense mutations in the DNA-binding domain of the *p63* gene on chromosome 3q27 have been associated with both EEC syndrome and non-syndromic split hand/foot malformation (SHFM). We have screened 25 SHFM and 7 EEC patients for mutations in *p63*. Among the SHFM patients, 12 had isolated SHFM and 11 had SHFM associated with long bone deficiency (SHFLD). The other 2 patients had syndromic SHFM, including one with nystagmus (Karsch Neugebauer syndrome) and one with seizures and mental retardation. All of the EEC patients displayed 2 or more characteristic features of EEC syndrome. Two of these patients also had a coloboma and another had seizures, mental retardation and blindness. SSCP analysis of *p63* detected no mutations in the DNA binding domain (exons 5-10) in the 25 SHFM patients. In 20 of these patients, additional SSCP analysis of exons 1-4 and 11-14 was performed and did not demonstrate any mutations. In the EEC patients, 4 missense mutations within the DNA binding domain were detected, including two novel mutations. Two of the EEC patients, neither of whom have clefting of the lip and/or palate, had a missense mutation affecting the G at position 755, G755T and G755A, leading to R204Q and R204P substitutions respectively. The other 2 patients, both of whom have clefting of the lip and/or palate, had a mutation affecting the G at position 1055, G1055A and G1055C, leading to R304Q and R304P substitutions respectively. These observations support a major causative role for *p63* in EEC syndrome but not in non-syndromic SHFM. In addition, the mutations identified in our EEC patients suggest a correlation between the position of the mutation within the DNA binding domain of *p63* and the presence/absence of cleft lip/palate.

**Molecular studies of preaxial polydactyly in three North American families and a novel mouse mutant.** A.V. Hing<sup>1</sup>, S. Cook<sup>2</sup>, M.B. Dobbs<sup>3</sup>, N. Syed<sup>1</sup>, M. Davisson<sup>2</sup>. 1) Div Gen/Dev, Univ Washington, Seattle, WA; 2) The Jackson Laboratory, Bar Harbor, ME; 3) Dept Orthopaedic Surg, Wash Univ, St. Louis, MO.

Preaxial polydactyly (PPD) with triphalangeal thumb is an autosomal dominant trait linked to human chromosome 7q36. Although PPD has been mapped to a region of 500 kb on chromosome 7q36, the molecular basis remains unknown. Linkage studies have expanded the phenotype to include complex polysyndactyly, tibial hemimelia, and sternal defects. Three mouse mutants, (*hemimelic extra-toes*, *luxate*, and *sasquatch*), have been mapped to the region of mouse Chromosome 5 that is homologous to the human chromosome 7q36 PPD locus. The phenotype in the mouse mutants is strikingly similar to the human counterpart including preaxial polydactyly, triphalangeal first digit, and tibial hemimelia. We describe a new mouse mutant, *preaxial polydactyly with hemimelia*, with preaxial polydactyly of all four limbs and tibial hemimelia. This mutant arose spontaneously in a C57BL/10 congenic called B10.PL-H2<sup>u</sup>(73NS)/Sn. Both homozygotes and heterozygotes are affected equally and both sexes appear to be viable and fertile. Linkage studies performed at The Jackson Laboratory demonstrated flanking markers of *D5Mit124* and *D5Mit229* at 13 cM and 18 cM from mouse Chromosome 5 centromere, respectively. There were no recombinants in 100 meioses with the marker *D5Mit387*, 15cM from the centromere, placing *preaxial polydactyly with hemimelia* close to *hemimelic extra-toes*. Three families with PPD linked to chromosome 7q36 are available for mutation studies. These include a family from Illinois segregating triphalangeal thumb with variably duplicated thumbs/great toes and two pedigrees from Iowa, one with isolated bilateral triphalangeal thumb. Genomic sequence for both the human and mouse PPD loci in the form of finished/unfinished sequence can be accessed from Genbank. We have identified regions of mouse/human sequence homology, aligned known human and mouse orthologous transcripts [*HLX9(Hlxb9)*, *c7orf2(Lmbr1)*, *FKSG33(Lmbr2)*, and *HFBck28 (D5Kng1)*], and characterized novel transcripts from the PPD region. We describe mutation analysis of candidate transcripts in both human pedigrees and mouse mutant.

**Mutations in the Cartilage Derived Morphogenetic Protein-1, in kindred affected with Grebe Type severe short limb dwarfism and DuPan Syndrome.** *M.F. Ul Haque<sup>1</sup>, W. Ahmad<sup>2</sup>, S.H.E Zaidi<sup>1</sup>, S. Haque<sup>2</sup>, A. Wahab<sup>2</sup>, A.C. Azim<sup>3</sup>, A.S. Teebi<sup>1</sup>, M. Ahmad<sup>2</sup>, D.H. Cohn<sup>4</sup>, T. Siddique<sup>3</sup>, L.C. Tsui<sup>1</sup>.* 1) Dept Genetics, Hosp Sick Childre, Toronto, ON, Canada; 2) Quaid-i-Azam Univ, Islamabad, Pakistan; 3) Northwestern Univ Med Sch, Chicago, IL; 4) Cedars-Sinai Med Cent, Los Angeles, CA.

Autosomal recessive inheritance was observed in two large inbred families from Pakistan exhibiting the clinical features resembling the Grebe type severe short limb dwarfism (13 affected) and fibular hypoplasia and complex brachydactyly similar to DuPan Syndrome (9 affected). While carrier parents did not exhibit any apparent skeletal abnormalities, affected individuals show reductions and absence of bones in the limbs, and appendicular bone dysmorphogenesis with unaffected axial bones in all affected individuals. Cartilage derived morphogenetic protein-1 (CDMP-1) has been reported to regulate limb patterning and distal bone growth. CDMP-1 is a member of the transforming growth factor- $\beta$  superfamily of secreted signaling molecules that regulate gene expression through interactions with the bone morphogenetic protein receptors and smad proteins. We examined the genomic DNA from affected and unaffected subjects of the two families for possible mutations in the CDMP-1 gene. In the family exhibiting Grebe type phenotype, an insertion of C at nucleotide 297 of the coding sequence was discovered. This insertion produced a shift in the reading frame at amino acid 99 causing truncation of the polypeptide at six amino acids downstream. This is expected to result in complete loss of its signaling function due to the absence of secreted active domain of CDMP-1. In the second family that we have previously reported, exhibited the clinical features of Du Pan syndrome, a missense Leu441Pro substitution was found in the mature domain of the CDMP-1 gene. This mutation may alter the activity of CDMP-1 protein and interfere with its downstream signaling. These changes were not found in the 41 control subjects of Pakistani origin. Our finding confirms that CDMP-1 regulates limb bones growth during development and further suggests that its absence does not interfere with other developmental processes.

**Alpha-B crystallin gene (CRYAB) mutation causes dominant congenital posterior polar cataract in man.** *P.J. Francis<sup>1</sup>, V. Berry<sup>1</sup>, M.A. Reddy<sup>1</sup>, D. Collyer<sup>1</sup>, E. Vithana<sup>1</sup>, I. MacKay<sup>3</sup>, G. Dawson<sup>3</sup>, A.H. Carey<sup>3</sup>, A. Moore<sup>1,2</sup>, R.A. Quinlan<sup>4</sup>, S.S. Bhattacharya<sup>1</sup>.* 1) Molec Genetics, Inst Ophthalmology, London, England; 2) Moorfields Eye Hospital, London, England; 3) Oxagen Limited, 91 Milton Park, Abingdon, Oxon, OX14 4RY, UK;; 4) Department of Cell Biology, University of Durham, Durham, UK.

Congenital cataract is the commonest treatable cause of childhood blindness in USA and Europe with a prevalence of 1-6 cases per 10,000 births. Inherited cataracts accounts for up to half of all congenital cataract and the commonest mode of inheritance is autosomal dominant (ADCC). To obtain further insight into the genetic aetiology of congenital cataract we performed linkage analysis on a four-generation family of English descent with autosomal dominant posterior polar cataract. Twelve affected and ten unaffected members of the family underwent a full ophthalmological examination, after ethical approval was granted. The opacity which, was bilateral in all cases, consisted of a single well-defined plaque confined to the posterior pole of the lens and varied from 0.5-3 mm in diameter. We identified a new ADCC locus on chromosome 11q21.2-q22.3. This locus (CPP2) was mapped to a 16cM region flanked by microsatellite markers D11S4176 and D11S908 encompassing the candidate gene CRYAB, which encodes aB crystallin. Sequence analysis of the CRYAB gene revealed a deletion mutation (450delA) in exon3 that co-segregated with disease in the family. The mutation causes a frameshift in codon 150 and produces an aberrant protein consisting of 184 residues. The mutation was not found in a panel of 100 normal unrelated individuals thus excluding the possibility that it was a rare polymorphism.

**Linkage analysis, physical mapping and candidate gene analysis of the Amish microcephaly (MCPHA) locus of 17q25.** *M.J. Rosenberg<sup>1</sup>, I. Makalowska<sup>1</sup>, G. Bouffard<sup>2</sup>, R. Agarawala<sup>3</sup>, J. Davis<sup>1</sup>, T. Koch<sup>4</sup>, J.L. Weber<sup>5</sup>, L.M. Kalikin<sup>6</sup>, E.M. Petty<sup>6</sup>, M.S. Hilliard<sup>7</sup>, D.H. Morton<sup>8</sup>, R.I. Kelley<sup>9</sup>, A.A. Schäffer<sup>3</sup>, L.G. Biesecker<sup>1</sup>.* 1) NHGRI/NIH, Bethesda, MD; 2) NISC/NIH, Bethesda, MD; 3) NCBI/NIH, Bethesda, MD; 4) Konrad-Zuse-Zentrum für Informationstechnik, Berlin; 5) Marshfield Med Res Foundation, Marshfield, WI; 6) U. Michigan Med Ctr, Ann Arbor, MI; 7) Logicon ROW Sciences Corp, Rockville, MD; 8) Clinic for Special Children, Strasburg, PA; 9) Kennedy-Krieger Inst, Baltimore, MD.

Amish microcephaly (MCPHA) is a distinct syndrome with severe congenital microcephaly, increased urinary excretion of the TCA cycle intermediate,  $\alpha$ -ketoglutarate, and premature death. The disorder is inherited in an autosomal recessive pattern and has only been observed in Old Order Amish families who have ancestors in Lancaster County, PA. Using a genealogy database and automated pedigree software, we constructed a pedigree connecting 21 sibships with an affected child to a common ancestor. A whole genome scan, fine mapping, and haplotype analysis localized the MCPHA gene to a 3 cM or 2 Mb region on 17q25. Six consecutive standard markers from D17S1301 to D17S801 are consistent with linkage and have two-point LOD scores above 10.0 using equal allele frequencies. A physical map of this region was constructed that contains 79 genomic clones of which 21 have sequence data in GenBank. The sequenced BACs have been assembled into three contigs. We have analyzed the contigs using the Santa Cruz Working Draft website and BLAST searches and found 27 characterized genes and 56 additional clusters of full-length transcripts or spliced ESTs. We have connected the three contigs derivable from the public sequence data into a single contig by STS mapping and are currently sequencing three BACs to obtain sequence information for the two gaps. We are analyzing the entire region using gene prediction software to look for additional candidate genes. We hypothesize that this disorder is due to a mutation in a gene involved in  $\alpha$ -ketoglutarate metabolism with secondary microcephaly, a mutation in a pleiotropic gene that affects brain development and the TCA cycle, or a recessive contiguous gene syndrome.

**Genetic Analysis of Two Pedigrees with Charcot-Marie-Tooth Neuropathy Type 1C.** *V.A. Street<sup>1</sup>, J.D. Goldy<sup>2</sup>, A.S. Golden<sup>1</sup>, B.L. Tempel<sup>1</sup>, T.D. Bird<sup>3</sup>, P.F. Chance<sup>2</sup>.* 1) Bloedel Hearing Ctr, Univ Washington, Seattle, WA; 2) Pediatrics, Division of Genetics and Development, University of Washington School of Medicine, Seattle, WA; 3) Neurology, University of Washington School of Medicine and Veterans Administration Medical Center, Seattle, WA.

Charcot-Marie-Tooth (CMT) neuropathy is the most common inherited peripheral nervous system disorder affecting approximately 1 in 2000 individuals and characterized by degenerative changes in motor and sensory nerves. The hallmark of CMT Type I (CMT1) is reduced nerve conduction velocities (NCVs) (<40 meters/sec) and nerve biopsies that demonstrate hypertrophic demyelination. The present study includes two large five-generation CMT1C pedigrees (K1550 and K1551). Affected members have clinical findings and reduced NCVs consistent with CMT1. Male-to-male transmission is present confirming autosomal dominant inheritance. Previous linkage analysis with markers from the CMT1A region on chromosome 17p11-12 and CMT1B region on chromosome 1p21 excluded linkage. Furthermore, the DNA duplication commonly associated with CMT1A is not present. Sequence analysis for five genes known to play critical roles in the development of demyelinating neuropathies, the peripheral myelin protein (PMP-22), the myelin protein zero (MPZ), and the early growth response 1 and 2 genes (EGR1 and 2) also known as Krox-24 and Krox-20, and PERP, disclosed no abnormalities, confirming further genetic heterogeneity in CMT1 and indicating that the mutant gene in these CMT1C pedigrees represents a novel unmapped form of CMT1. To assign a chromosomal address, we are performing a 10 cM genome scan on both pedigrees. No linkage has been detected on chromosomes 5, 6, 14, and 19-22 for pedigree K1550, nor on chromosomes 19-22 for pedigree K1551.

**A recessive contiguous gene deletion of chromosome 2p16 associated with cystinuria and a mitochondrial disease.**

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Deletions ranging from a 100Kb to a megabase, which are too small to be detected under the microscope, may still involve dozens of genes, thus causing microdeletion syndromes. The vast majority of these syndromes are caused by haploinsufficiency of one or several genes and are transmitted as dominant traits. We identified seven patients originating from an extended family who presented with a unique syndrome, inherited in a recessive mode, characterized by cystinuria, neonatal seizures, hypotonia, lactic acidemia, severe somatic and developmental delay and facial dysmorphism. Reduced activity of all the respiratory chain enzymatic complexes which are mitochondrial encoded was found in the muscle biopsies of the patients examined. The molecular basis of this disorder is a homozygous deletion of 179,311 bp on chromosome 2p16, which includes the type I cystinuria gene SLC3A1, the protein phosphatase 2C beta gene, an unidentified gene (KIAA 0436) and several ESTs. The extent of the deletion suggests that this unique syndrome is related to the complete absence of all these genes products, one of which may be essential for the synthesis of mitochondrial-encoded proteins.



***FOXC2* truncating mutation in distichiasis lymphedema and cleft palate (DLC).** *M. Bahuau*<sup>1,2</sup>, *V. Soupre*<sup>2</sup>, *R. Couderc*<sup>1</sup>, *M-P. Vazquez*<sup>2</sup>, *C. Houdayer*<sup>1</sup>. 1) Biochimie et Biologie Moléculaire, Hôpital Trousseau, Paris, France; 2) Chirurgie Maxilo-Faciale, Plastique et Stomatologie, Hôpital Trousseau, Paris, France.

We have previously reported a family showing autosomal dominant segregation of upper- and lower-eyelid distichiasis (double row of eyelashes) in seven affected relatives over three generations, in addition to below-knee lymphedema of pubertal onset (lymphoedema praecox) in three of them. Two children had cleft palate/velum in addition to distichiasis, and without the previously reported association to the Pierre Robin anomalad. Other ophthalmologic anomalies included divergent strabismus and early-onset myopia. This family was found to be thoroughly linked to D16S3074 and thereby proposed to be allelic to distichiasis-lymphedema syndrome (DL, MIM 153400) in 16q24.3, although pterygium colli, congenital heart disease, or facial dysmorphism were not features here. Since *FOXC2/FKLH14* mutations were found to underlie DL and diverse hereditary lymphedema conditions, this gene was thoroughly scanned by sequence analysis. An out-of-frame deletion (914-921del) was identified and found to segregate with the disease, further highlighting the phenotypic heterogeneity of lymphedema conditions linked to *FOXC2* truncating mutations. Whether such heterogeneity is related to genotype-phenotype correlation, a hypothesis not primarily supported by the apparent loss-of-function mechanism of the mutations, or governed by modifying genes, remains to be determined.

**Phenotypic heterogeneity in lymphedema-distichiasis with *FOXC2* mutations.** *T.W. Glover<sup>1</sup>, R.P. Erickson<sup>2</sup>, S.L. Dagenais<sup>1</sup>, M.T. McDonald<sup>3</sup>, M.S. Caulder<sup>1</sup>, B.P. Brooks<sup>4</sup>, M.W. Glynn<sup>1</sup>.* 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Pediatrics, University of Arizona, Tucson, AZ; 3) Department of Pediatrics, Duke University, Durham, NC; 4) Department of Ophthalmology, University of Michigan, Ann Arbor, MI.

Hereditary lymphedema-distichiasis is an autosomal dominant disorder that classically presents as lymphedema of the limbs, with variable age of onset, and aberrant growth of eyelashes along the lid margins and from the Meibomian glands (distichiasis). Other reported complications include cardiac defects, cleft palate, photophobia, hydrops and extradural cysts. Recently, we reported that truncating mutations in the forkhead transcription family member *FOXC2* resulted in lymphedema-distichiasis in two families. We now describe an additional eight families with lymphedema-distichiasis, including the original family reported by Falls and Kertesz in 1964. Through sequence analysis, we identified truncating mutations in all families studied. These included 3 nonsense, 3 insertion, 1 deletion, and 1 insertion-deletion mutation. Distichiasis was the most common clinical feature, followed by age-dependent lymphedema. Distichiasis alone was found in one small pedigree ascertained for this disorder. There is large variation in expression of associated secondary features with tetralogy of Fallot and cleft palate each seen in about 10% of cases. Our results are consistent with the interpretation that haploinsufficiency for *FOXC2* is responsible for the phenotype in all cases. There is no apparent genotype-phenotype correlation. The factors that determine phenotypic heterogeneity are at present unclear, but are important for characterization of the molecular mechanisms underlying lymphedema-distichiasis and for informative genetic counseling.

**In vitro expression studies of 11 mutations identified in the GLB1 gene of GM1- gangliosidosis patients. A.**

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The deficiency of acid b-galactosidase results in two lysosomal storage disorders: GM1 gangliosidosis (infantile, juvenile and adult forms) and Morquio B. GLB1 gene encodes for two proteins: GLB1 and EBP. We have previously identified 11 aminoacid substitutions in 8 GM1 gangliosidosis patient. The S54N, R59H, R59C, G579D, Y591C and the new P263L were detected in 4 patients affected by the infantile form, the R201H and C230Y were found in the 2 juvenile patients and the T329A, R442Q and R521C were detected in 2 adult patients. Only the R201H mutation was previously expressed in vitro. In order to determine the impact of each mutation on the biochemical properties of the normal enzyme we carried out expression studies in COS-1 cells. GLB1 enzymatic assays showed that the mutations: S54N, R59H, R59C, P263L, Y591C, G579D, C230Y and T329A rendered b-galactosidase enzymatically inactive. The R201H, detected in compound heterozygosity in the 2 juvenile patients, and the R442Q, identified in one adult patient, resulted in residual activity of the mutant enzymes, accounting for the mild clinical phenotype of the patients. Expression of the R201H mutant resulted in a level of GLB1 activity higher than that of the R442Q variant. This finding can be due to differences in affinity of the two mutant proteins towards the synthetic substrate. The R521C genetic lesion, previously reported as a polymorphism, was detected at homozygous state in one adult patient. Expression study, showing an enzymatic activity of 51% of normal values, suggested a possible effect of the R521C on the GLB1 activity. Except for the R201H and the C230Y, all the mutations studied can affect both GLB1 and EBP. Each mutation could interfere with the processing of the precursors, the transport of mutant GLB1 to the lysosome, the capacity of b-galactosidase to assemble with PPCA and neuraminidase and/or the kinetic properties and activity of the mutant enzymes. The financial support of Telethon-Italy and MPS-Italy are gratefully acknowledged.

**Screening of NCL genes in French patients with ceroid lipofuscinoses..** *C. Caillaud*<sup>1,2</sup>, *J.P. Puech*<sup>1,2</sup>, *J. Manicom*<sup>1,2</sup>, *L. Poenaru*<sup>1,2</sup>. 1) Dept Genetique, CHU Cochin, Paris, France; 2) INSERM U129, CHU Cochin, Paris, France.

Neuronal ceroid lipofuscinoses (NCL) are inherited neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigments in various tissues. Four main NCL forms are distinguished according to clinical and morphological features : infantile (INCL), late infantile (LINCL), juvenile (JNCL) and adult (ANCL), but numerous atypical forms have also been described. Recent advances in the molecular genetics of NCL has allowed to delineate at least eight loci (from CLN1 to CLN8) as responsible for these different forms, two of them encoding soluble enzymes : palmitoyl-protein thioesterase (PPT) for CLN2 and tripeptidyl-peptidase I (TPP-I) for CLN1. In LINCL, the most common clinical form in France, the CLN2 locus was frequently involved, as demonstrated by the presence of a tripeptidyl-peptidase I deficiency in the majority of patients. Complete sequencing of the CLN2 exons showed the predominance of two previously reported mutations (Arg208Stop and 3556 G>C), accounting for 28 and 34 % of the alleles, respectively. Novel private mutations were also characterized, such as deletions (6082-6092del), splice (4635 A>G), nonsense (Trp548Stop, Gln509Stop, Glu394Stop) or missense (Arg339Trp, Ser153Pro, Ser456Pro) mutations. Some LINCL patients had no TPP-I deficiency and are likely candidate for the CLN6 or CLN7 loci. A palmitoyl-protein thioesterase (PPT) deficiency was found in three classical INCL patients with age of onset between 7 and 20 months and in one patient with an early juvenile form. Four previously reported CLN1 mutations were characterized in these patients : Lys55Stop, 774-775insA, Arg151Stop, Val181Met. Two novel mutations were also described : Gln142Stop and Tyr109Cys, which one was found in the patient presenting the early juvenile form. Among patients with classical juvenile forms, only 3 alleles were found to carry the common 1.0 kb deletion. Further studies will define if other mutations of the CLN3 locus are responsible for these late-onset forms or if various other genes are involved in these patients. Delineation of the NCL loci will allow to offer a reliable genetic counselling to couples at-risk for these diseases.

**Fabry disease: identification of ten novel mutations.** *K. Azibi<sup>1,2</sup>, C. Caillaud<sup>1,2</sup>, J. Manicom<sup>1</sup>, J.P. Puech<sup>1</sup>, A. Kahn<sup>2</sup>, L. Poenaru<sup>1,2</sup>.* 1) Genetique, Faculte de Medecine Cochin, Paris, France; 2) INSERM U-129, CHU Cochin, Paris, France.

Fabry disease is an X-linked glycosphingolipid storage disease caused by a deficiency of the lysosomal enzyme alpha-galactosidase A (GLA). This deficiency leads to the accumulation of uncleaved glycosphingolipids in the lysosomes of vascular endothelial and smooth muscle cells and also in plasma. Patients studied have typical clinical manifestations of Fabry disease, including angiokeratoma, abdominal pain, corneal opacities, paresthesias in the extremities and renal failure. All of them exhibited an alpha-galactosidase deficiency, with a GLA residual activity comprised between 0.8 and 4 nmol/h/mg protein. To clarify the molecular mechanism causing the enzyme defect and to facilitate a rapid detection of Fabry hemizygotes and carriers, the seven GLA exons, including intron-exon junctions were PCR-amplified and directly sequenced. 14 mutations were characterized in this group of patients, among which four have been previously reported. Ten novel mutations were identified, including two nonsense mutations (W245X and W262X), three missense mutations (A156N, Q279H, S297C), a small nucleotide insertion (insG10678) and four small deletions (1235del15, 7316delA, 7337delG, 10666 delATCA) resulting in premature stop codon. These mutations were found in 18 Fabry patients and further helped the heterozygosity detection in carrier females. These results demonstrate the molecular heterogeneity of Fabry disease. The study also permit to explore possible genotype / phenotype correlations in patients with previously described mutations. Finally, the origin of mutational events in different families will be precised by the haplotype determination for some mutations, using microsatellite polymorphisms in the alpha-galactosidase A gene.

**Identification of novel mutations in the cystinosin gene (CTNS) in patients with cystinosis.** *M. Kiehntopf<sup>1</sup>, H.G. Koch<sup>2</sup>, T. Coskun<sup>3</sup>, S. Ozen<sup>3</sup>, T. Deufel<sup>1</sup>, E. Harms<sup>2</sup>.* 1) Institut fuer Klinische Chemie, Friedrich-Schiller Universitaet Jena, Jena, Germany; 2) Klinik und Poliklinik fuer Kinderheilkunde, Universitaetsklinikum Muenster, Muenster, Germany; 3) Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Turkey.

Cystinosis is an autosomal recessive lysosomal storage disorder caused by mutations in the Cystinosin (CTNS) gene; CTNS consists of 12 exons and encodes a 55 kDa lysosomal transmembrane protein. To date, 46 different CTNS mutations have been described in the most common form of cystinosis, the classical, infantile nephropatic phenotype; non-classical, benign, intermediate or the late-onset form of the disease have been associated with, so far, nine distinct CTNS mutations. In a population of European cystinosis patients we have analyzed the CTNS gene by sequencing of exons 3 to 12. We have identified six novel CTNS mutations, including one deletion involving exon 3 (400delGGT) resulting in disruption of the 5'-splice site in intron 3, and two insertions in exon 6 (632insA) and exon 7 (684insCACTT) leading to a frame shift with a premature stop at codon 124 and 119, respectively. Moreover, we have identified three novel nucleotide substitution in exon 11 (1262G>T), exon 7 (790A>G) and exon 10 (1118 T>C) leading to an amino-acid change at codon 308 (G>V) in the predicted transmembrane domain TM6, at codon 151 (R>G) and at codon 260 (I>T), respectively.

**Novel mutations and haplotype analysis in a-mannosidosis.** *H.M.F. Riise Stensland*<sup>1, 2</sup>, *H. Klenow*<sup>2</sup>, *D. Malm*<sup>3</sup>, *O.K. Tollersrud*<sup>4</sup>, *O. Nilssen*<sup>2</sup>. 1) Department of Human Genetics, Gonda #6309, UCLA, Los Angeles, CA, USA; 2) Department of Medical Genetics, University Hospital of Tromsø, Tromsø, Norway; 3) Department of Internal Medicine, University Hospital of Tromsø, Tromsø, Norway; 4) Department of Medical Biochemistry, University of Tromsø, Tromsø, Norway.

Lysosomal a-mannosidase (LAMAN, EC 3.2.1.24) is an exoglycosidase involved in the ordered degradation of N-linked oligosaccharides. Lack of LAMAN activity leads to the lysosomal storage disorder a-mannosidosis (MIM 248500), an autosomal recessive disorder described in man, cattle, cats and guinea pigs. Affected individuals accumulate partially degraded oligosaccharides in the lysosomes, and typical symptoms in man are mental retardation, hearing loss, recurrent infections and various skeletal changes. We have earlier reported 23 disease causing mutations in the LAMAN gene of 42 unrelated a-mannosidosis patients. Here we have analyzed additional 43 unrelated patients by screening for known mutations and by sequencing of the 24 LAMAN exons as well as the exon-intron borders. Thirty-three novel mutations; 9 missense, 8 nonsense, 5 splice site and 11 small insertions/deletions, were identified. In total, the 56 mutations were detected on 159 of the 170 alleles (93.5%). Most of the mutations were private or occurred in 2-3 families, except for one missense mutation, R750W, that was detected in patients from 12 different countries, and present on 55 unrelated alleles (32.3%). Haplotype analysis using 4 intragenic SNPs revealed that the 750W allele existed on 3 different haplotypes. The majority of the alleles where the phase could be determined (36 of 40, 90%) shared the same haplotype, indicating that the 750W mutation probably spread by migration and founder effect. The 2 other associated haplotypes could have emerged from the ancestral haplotype by single recombinatorial events or, alternatively, resulted from recurrent mutational events.

**Towards identification of the mental retardation genes associated with balanced chromosome translocations.** *P.S. Edwards<sup>1</sup>, K.E. Miller<sup>1</sup>, M. Beachem<sup>1</sup>, S. Ladd<sup>1</sup>, F. Romero-Pastrana<sup>1</sup>, R.J. Schroer<sup>1</sup>, K. Clarkson<sup>1</sup>, L. Seaver<sup>1</sup>, R. Saul<sup>1</sup>, R.C. Rogers<sup>1</sup>, G.S. Pai<sup>2</sup>, C.E. Schwartz<sup>1</sup>, B.R. DuPont<sup>1</sup>, R.E. Stevenson<sup>1</sup>, A.K. Srivastava<sup>1</sup>.* 1) J C Self Res Inst of Hum Genet, Greenwood Genetic Ctr, Greenwood SC; 2) Dept of Pediatrics, Med Univ of South Carolina, Charleston, SC.

Mental retardation (MR) is a common condition affecting about 2-3% of the human population. Among various causative factors, a substantial portion of the genetic component of MR is due to mutations in specific genes. Subtelomeric deletions account for approximately 6% of idiopathic MR. Several syndromic and non-syndromic MR genes (predominantly X-linked) have been cloned, and in many cases MR-associated balanced chromosome translocations have been instrumental in the MR gene identification. Mapping and cloning of autosomal MR gene remains difficult due to the lack of large families and the phenotypic variability among small families precludes the pooling of families for linkage analysis. We have identified fifteen patients with balanced translocations and MR. Clinically most of the patients have profound MR and in many cases, additional abnormalities have also been noted. Cytogenetic characterization and preliminary molecular analysis of the translocation breakpoints in seven MR patients with autosome; autosome translocation, and in two female MR patients with X; autosome translocation, have delineated several potential chromosomal regions for autosomal and X-linked MR. FISH analysis using genomic clones to map the translocation breakpoints has further narrowed the critical MR regions to less than 3 Mb in the first seven cases analyzed. A BAC clone spanning the Xq24 breakpoint in one female patient has been identified and is being characterized for the candidate MR gene. In a second patient the Xp21.2 breakpoint was found to disrupt the DMD gene. A cryptic deletion at a 6q22 breakpoint has been found in one patient. Identification and characterization of the candidate MR genes associated with these translocation should provide a major advance in understanding brain function critical for development of intellectual and learning abilities as well as facilitating objective diagnosis in a portion of the MR populations.



**Maternally-transmitted MECP2 gene deletion in a 4-year-old boy with cognitive impairment and developmental delay.** *A. Bottani<sup>1</sup>, A. Dayer<sup>1</sup>, D. Cruzado<sup>2</sup>, C.-A. Haenggeli<sup>2</sup>, S.E. Antonarakis<sup>1</sup>, M.A. Morris<sup>1</sup>.* 1) Div Medical Genetics, Dept Gynecology/Obstetrics, Geneva Univ Hosp, Geneva, Switzerland; 2) Neuropediatric Unit, Dept Pediatrics, Geneva Univ Hosp, Geneva, Switzerland.

Mutations of MECP2, the X-linked gene responsible for Rett syndrome in girls, have also been recently implicated in males presenting with variable clinical pictures, ranging in severity from lethal infantile encephalopathies to non-specific mental retardation in adult patients.

We report a mentally-handicapped 4-year-old boy and his unaffected transmitting mother, both having a 44 bp truncating deletion (1158del44) in exon 4 of MECP2, resulting in a frameshift and premature truncation (386fs388X) of the MECP2 protein.

The proband was born at term after a pregnancy marked by diminished fetal movements and growth retardation. Birth weight and head circumference were below the 10th centile, while length was at the 10th centile. The baby was placid and showed axial hypotonia. Milestones were delayed: sitting at 10 months, standing with help at 15 months, first words at 20 months. Independent walking has never been achieved. Facial phenotype showed low-set anteverted ears and a prominent frontal upswing ("cowlick") of hair. Motor regression was noted at the age of 46 months, the child being unable to crawl or stand anymore. Seizures (up to 20 episodes/day) characterized by tonic upper limb movements, eye revulsion and loss of contact were observed. EEG showed diffuse paroxysmal abnormalities. Neurological examination at 4 years revealed microcephaly, involuntary stereotypic hand movements and frequent hand-to-mouth movements, intentional tremor, permanent head nodding, intermittent convergent strabismus and multidirectional nystagmus, bruxism, absent speech, spasticity of the inferior limbs with hyperreflexia and a bilateral Babinski sign.

MECP2 mutations must now be considered in the diagnostic work-up of males with unexplained mental retardation and/or neurological regression.

**X linked recessive syndrome with severe mental retardation, spastic dystonia, seizures, and migraine.** *K.L. Gardner<sup>1</sup>, N. Pulipaka<sup>1</sup>, T. Wieser<sup>1,2</sup>, M. Barmada<sup>1</sup>, R. Hendrickson<sup>1</sup>, E. McPherson<sup>3</sup>, N. Bohnen<sup>1</sup>.* 1) Neurology, Human Genetics Univ Pittsburgh, Pittsburgh, PA; 2) Neurology Martin-Luther University of Halle, Germany; 3) Genetics, Magee Women's Hospital, Pittsburgh, PA.

Several X-linked MR syndromes (MRXS) with different extrapyramidal features have been described, including MR with Parkinsonism, psychosis, macroorchidism at Xq28; Parkinsonism, seizures, megalencephaly at Xq27.3-q28; dystonic hand movements at Xp22; and dystonia, optic atrophy, deafness at Xq22. Clinical evaluation included Stanford Binet testing, neurologic exams, videos. Blood for DNA extraction / cell lines was collected from 45 members. Twenty-five fluorescent labeled markers spanning ChrX were genotyped and two-point, multipoint linkage, affected relative allele sharing performed using VITESSE 1.1 and ALLEGRO with Sall scoring function. We examined 37/45 available family members from 4 generations including 5/13 affected males with severe MR, spastic-dystonia, dysarthria, history of seizures (2), headaches (3), later onset loss of ambulation with contractures, mild scoliosis (1), normal stature, near normal life span. The most severely affected patient has macrocephaly but proband has microcephaly and macroorchidism without definite dysmorphic features. Five obligate carriers have variable cognitive impairment with dementia noted by sixth decade. Migraine was prominent in females (16/18) without aura (7) and with aura (11) including hemiparesis (3 branches) but not characterized for MR males. Proband had normal karyotype, metabolic and SCA screen, with MRI showing mild cerebellar atrophy. Scores for MR-dystonia peaked at Xq28, DXS8061 with multipoint LOD 1.67, theta 0 and NPL 5.9 using 65% penetrance, 0% phenocopy, allele freq .0001. Highest scores for migraine peaked more proximally at DXS1001, Xq24 showing 2-point LOD 1.12, theta 0, co-dominant model with penetrance 75-90%, .01% phenocopy, and 20% allele freq. This may represent a new MR syndrome with distinctive extrapyramidal features and suggestion of linkage at Xq28. High prevalence of migraine in this family may be partly due to pleiotropy and variable expression of the MRXS gene plus additional X linked or autosomal contributions.

**Mutation analysis of EXT1 and EXT2 genes in two patients with hereditary multiple exostoses and mental retardation.** *H. Li, T. Yamagata, M. Mori, M.Y. Momoi.* Department of Pediatrics, Jichi Medical School, Minamikawachi, Tochigi, Japan.

Hereditary multiple exostoses (EXT) is an autosomal dominant bone disorder that affects endochondral proliferation. EXT1 and EXT2 genes are responsible for EXT. EXT1 and EXT2 form a complex that catalyzes the synthesis of heparan sulfate. Mental retardation is reported in patients with EXT and tricho-rhino-phalangeal syndrome (TRPS) as a contiguous gene deletion syndrome of EXT1 and TRPS1. The gene responsible for mental retardation has not been identified. Here we report two patients with severe EXT and mental retardation without TRPS. We screened the gene mutation of EXT1 and EXT2 and detected two base deletions in EXT1 in both patients. Both patients have mental retardation with pervasive developmental disorder. Their fathers also have EXT with normal intelligence. The patients EXT is more severe with early onset and many tumors than their fathers. Their mothers were unaffected. We detected the mutations of EXT1 and EXT2 genes using DHPLC and direct sequencing analysis in both patients. Patient I had a 1742delTGT-G in the exon 9 of EXT1 gene. Patient II had a 2093delTT in the exon 11 of EXT1 gene. These two deletions were both inherited from their fathers and caused frame-shift and early termination of EXT1. EXT1 gene shows ubiquitous expression pattern including the brain. Here we propose that EXT1 is responsible for mental retardation and pervasive developmental disorder probably relating to the difference of the penetrance between the generation and the type of mutation, like tuberous sclerosis and neurofibromatosis. EXT1 may play an important role in cognitive function in the brain as well as tumorigenesis in the bony tissue.

**Complex Glycerol Kinase Deficiency: Defining the Deletional Breakpoints in the Original Patient Described with this Contiguous Gene Syndrome.** *K.K. Niakan<sup>1</sup>, Y.-H. Zhang<sup>2</sup>, B.-L. Huang<sup>2</sup>, E.R.B. McCabe<sup>2</sup>*. 1) Departments of Human Genetics and; 2) Pediatrics, UCLA, Los Angeles, CA.

We have previously reported a patient (CM) with Duchenne muscular dystrophy (DMD), glycerol kinase deficiency and adrenal hypoplasia congenita (Guggenheim et al, *Ann Neurol* 7:441, 1980). Subsequently we showed a less than 2Mb deletion in this patient (McCabe et al, *Am J Hum Genet* 51:1277, 1992). Southern analysis of genomic DNA from CM, using DMD probes, revealed that the deletion in the patient resided in a region within 1kb of the 3' end of the DMD mRNA (McCabe et al, *J Clin Invest*, 83:95, 1988). The goal of this investigation was to sequence the DNA breakpoints in CM and attempt to determine events responsible for the deletion in this Xp22-p21 region. The approach we used to define the breakpoint in CM involved obtaining the genomic DNA sequence for the breakpoint regions from the public and private databases in order to create PCR primers, which we used to walk from intact DNA toward each breakpoint in the patient. We have narrowed the region of the breakpoints to within approximately 300 base pairs on either side. The telomeric breakpoint is in intron 6 of a gene, interleukin-1 receptor accessory protein-like-1 (IL1RAPL1), which when mutated is associated with X-linked mental retardation (Carrie et al, *Nat Genet* 23:25, 1999). The centromeric breakpoint is in intron 71 of DMD. The deletion breakpoints observed in CM are very similar to those described by Jin et al (*Euro J Hum Genet* 8:87, 2000): telomeric in IL1RAPL1 intron 7 and centromeric in DMD intron 78. The involvement of IL1RAPL1 in these patients provides an explanation of their mental retardation. In addition, the similarities between their breakpoints suggest that there may be structural features in these regions that predispose to instability. Defining the exact nature of patient breakpoints will be important to not only elucidate the specific events involved in this particular human contiguous gene syndrome, but also to understand the mechanisms responsible more broadly for genomic instability.

**High frequency of *MECP2* mutations MR males: implications for routine DNA diagnostics.** *H.G. Yntema, T. Kleefstra, A.R. Oudakker, A. Jacobs, B.A. de Vries, E. Sijdermans, H.G. Brunner, B.C.J. Hamel, H. van Bokhoven.* Dept Human Genetics 417, Univ Medical Centre Nijmegen, Nijmegen, Netherlands.

Recently it was discovered that mutations in the methyl-CpG binding protein 2 (*MECP2*) gene are not always lethal in males. *MECP2* mutations have been found in males with a phenotype varying from severe mental retardation with, RETT-like, progressive neurological symptoms to mild nonspecific mental retardation. A recent French pilot study showed that the frequency of mutations in *MECP2* in mentally retarded males is comparable to the frequency of the CGG expansion in the *FMR1* gene. We have extended this study by screening the *MECP2* gene in a cohort of 500 male patients and 100 female patients who were negative for the expansions across the *FRAXA* CGG repeat. Our results confirm a mutation frequency of almost 2% in the Dutch population. To rule out the possibility of private polymorphisms, family members of the patients and a control population are being tested. The patients with a causal mutation will be clinically re-examined, to detect a possible phenotypic pattern. The results from this study will be presented. The high frequency of *MECP2* mutations in the Fragile-X negative population favours the implication of this gene in a routine diagnostic setting. This will greatly enhance the possibilities for diagnosis and counseling in MRX.

**Candidate genes for X-linked mental retardation and X-linked congenital ataxia on chromosome Xp11.22-**

**Xq13.3.** *K. POIRIER*<sup>1</sup>, *R. ZEMNI*<sup>1</sup>, *J.P. FRYNS*<sup>2</sup>, *H. VAN BOCKHOVEN*<sup>3</sup>, *H.H. ROPERS*<sup>4</sup>, *C. MORAINÉ*<sup>5</sup>, *E. BERTINI*<sup>6</sup>, *C. BELDJORD*<sup>1</sup>, *J. CHELLY*<sup>1</sup>, *T. BIENVENU*<sup>1</sup>. 1) INSERM U129, ICGM, CHU Cochin, Paris, France; 2) Center for Human Genetics, Department of Clinical Genetics, Leuven, Belgium; 3) Department of Human Genetics, University Hospital, Nijmegen, The Netherlands; 4) Max Planck Institut für Molekulare Genetik, Berlin-Dahlem, Germany; 5) Service de Genetique, CHU Bretonneau, Tours, France; 6) Department of Neurosciences, Bambino Gesù Children's Hospital, Rome, Italy.

Different disease entities such as X-linked mental retardation (XLMR) and congenital ataxia (XCA) have been mapped to the Xp11.22-Xq13.3 region. To identify the transcribed DNA sequences mapped within this large region, we searched the public databases. 53 known genes and 24 mRNAs or ESTs have been mapped within this 26 Mb region. We analysed the expression of 54 transcripts by RT-PCR on total RNA extracted from normal human fetal brain and lymphocytes. 45 genes were expressed in the brain of which either 7 showed no expression in lymphocytes. 5 further genes were only expressed in lymphocytes and 4 potential genes were not expressed in tissue. To date, in order to isolate genes involved in XLMR and XCA, the complete genomic organization of 3 candidate genes (KIAA0189, EPLG2 and AK001475) were defined by the computational analysis of cDNA and genomic DNA sequences. KIAA0189 was classified in the RhoGAP gene family; EPLG2 is a member of the Eph receptor family; and AK001475 was classified in the GTPase gene family. We analysed the coding sequence of these 3 candidate genes in 28 unrelated patients with XLMR (n=25) or XCA (n=3). DHPLC analysis was carried out using the Transgenomic system. The screening of KIAA0189 revealed three exonic polymorphisms R327Q, G425R and S529S. Analysis of the coding region of EPLG2 revealed the presence, in one of the patients with MR, of a missense mutation located in exon 4, V189A. This sequence variation was not found in more than ninety control X chromosomes. Other than an intronic polymorphism 1038+20C->T, no mutations were identified in AK001475. Further genes isolated from this region will also be evaluated for mutations in XLMR or XCA patients.

**Mutations Found in PKD1 by DHPLC Analysis.** *P.R. Fain<sup>1</sup>, W.J. Kimberling<sup>1</sup>, G. Stetler<sup>1</sup>, C. Roberts<sup>1</sup>, A. Johnson<sup>1</sup>, G. Wu<sup>2</sup>, S. Morrow<sup>2</sup>, M.L. Robinson<sup>2</sup>.* 1) Barbara Davis Ctr Childhd Diab, Univ Colorado Health Sci Ctr, Denver, CO; 2) Applied Genomics and Molecular Genetics, Transgenomic, Inc., Omaha, NE.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary diseases in man and is responsible for about 8-10% of all end-stage renal disease. The majority (85%) of cases are due to mutations in the PKD1 gene on chromosome 16p13.3. The PKD1 gene, which was isolated in 1994, spans about 52 kb of genomic DNA and consists of 46 exons that code for a 14 kb transcript. The predicted polycystin-1 protein has 4,302 amino acid residues. PKD1 mutation screening and studies of genotype/phenotype correlations have been impeded by the complex structure of the gene, much of which (~70%) is replicated at least 3 times in regions centromeric to the PKD1 gene.

We have designed and optimized 60 amplicons for the 46 exons of the PKD1 gene for DHPLC analysis. We are presently screening a population of 700 patients, and preliminary results indicate the presence of nucleotide changes.

**Coupled Analysis of DHPLC and Single-Base Extension to Localize and Genotype Mutations in the HFE Gene.**

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A powerful analytical method combining the ability to discover and genotype mutations linked to Hereditary Hemochromatosis (HH) was developed for the HFE gene. HH is a common autosomal recessive disease that affects ~1 in 300 persons of Northern European descent, with an estimated carrier frequency of 1 in 10. Excessive iron deposition in major body organs due to the loss of regulation of dietary iron absorption can lead to liver cirrhosis, diabetes mellitus, cardiomyopathy, endocrine dysfunctions, and arthropathy. Early diagnosis of this disorder is critical, since simple phlebotomy treatments to remove iron-rich red blood cells can result in a normal life expectancy for HH patients.

We developed denaturing-HPLC (DHPLC) methods to scan HFE exons 2, 4 and 5 for mutations. Samples that showed a heteroduplex pattern were analyzed using a multiplexed single-base extension (SBE) genotyping assay. This rapid and accurate method is capable of genotyping the following six missense mutations in the HFE gene: H63D, S65C, G93R, I105T, C282Y, and R330M. SBE primers were designed to anneal adjacent to the base pair of interest. A thermostable DNA polymerase was used to specifically extend the 3' end of the SBE primer with the 2'-3' dideoxynucleotide (ddNTP) that is the Watson-Crick complement of the target base to be genotyped. The SBE products were analyzed using ion-pair reversed-phase HPLC (IP-RP HPLC) on the Transgenomic Wave® Nucleic Acid Fragment Analysis System with the DNASep® column. IP-RP HPLC is able to reproducibly separate oligonucleotides which differ only at their 3' base position, with the 3' base elution order of C-G-A-T at a column temperature of 70°C. This approach combines the efficiency of DHPLC mutation scanning with the specificity of the SBE assay and can be readily adapted for a variety of applications in molecular diagnostics and SNP studies.



**Evaluation of real-time quantitative PCR for carrier detection of dystrophin gene rearrangements.** *S. Tuffery-Giraud, C. Saquet, S. Chambert, M. Claustres.* Lab Genetique Moleculaire, CHU Montpellier, France.

Large deletions and duplications in the dystrophin gene are detected in about two third of patients with Duchenne (DMD) or Becker (BMD) muscular dystrophy. This enables accurate carrier detection and prenatal diagnosis in those families. Diagnosis of heterozygous deletions is mainly based on haplotype analysis, involving detection of loss of heterozygosity for microsatellite alleles or other polymorphic markers, whereas Southern blot is the method currently used to assess gene dosage for duplications. To meet the need for an easy-to-perform, rapid direct diagnosis of BMD/DMD carrier status, we report the development of a method based on measurement of gene copy number. Gene copy number analysis by real-time quantitative PCR can be targeted directly to a selected sub region of the gene of interest, and does not depend on the availability of informative flanking markers or parental DNA. Also, it involves no radioisotopes and requires no post-PCR handling. Heterozygosity for duplication increases the gene dose from two to three, whereas heterozygosity for a deletion reduces the gene dose from two to one. The amplification was performed in the Light-Cycler<sup>TM</sup> PCR system using the LightCycler FastStart DNA master SYBR Green I kit. In order to demonstrate the accuracy and reliability of the method for genetic testing, we analyzed 15 patients and relatives females with previously identified deletions or duplications including either exon 11 or 44. Fifteen normal samples were also tested as controls. All samples were run in triplicate. In order to correct for differences in the amount of input genomic DNA, the method involves amplification of a reference locus with known copy number and the use of a common standard DNA for amplification of the test and reference loci. The presence or absence of the genomic deletion or duplication was unambiguously diagnosed in all individuals. Hence, our initial data demonstrated the effectiveness of this approach, which could be extended to the analysis of multiple dystrophin exons in a cost- effective manner. Support: the Association Franaise contre les Myopathies (AFM).

***GNAS1* loss-of-methylation defect in a PHP-Ib kindred with linkage discordance to the chromosomal region**

**20q13.3.** *M. Bastepe*<sup>1</sup>, *D. Feig*<sup>2</sup>, *H. Jüppner*<sup>1,3</sup>. 1) Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA; 2) Endocrinology and Metabolism, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) MassGeneral Hospital for Children, Massachusetts General Hospital, Boston, MA.

Pseudohypoparathyroidism type-Ib (PHP-Ib), a disorder characterized by isolated PTH resistance in the kidney, links to a region on 20q13.3 that includes a part of the imprinted *GNAS1* locus. The autosomal dominant form of PHP-Ib becomes clinically apparent only after maternal transmission of the mutated allele, and is associated with a loss of methylation at *GNAS1* exon A/B. Current evidence thus suggests that the genetic defect causing PHP-Ib resides within close proximity of *GNAS1*, presumably disrupting a cis-acting element that regulates imprinting of this locus. We investigated a novel PHP-Ib kindred in whom a loss of methylation at exon A/B was demonstrated for the two affected individuals, but not the unaffected family members. These findings were similar to those previously reported for several sporadic and familial cases of PHP-Ib. However, analysis of markers spanning the PHP-Ib locus on 20q13.3 (~9 cM) revealed that the haplotype shared by the two affected sisters was also present in an unaffected sibling and an unaffected daughter/niece. Considering autosomal dominant inheritance, these data indicate linkage discordance between the genetic defect responsible for the PTH-resistance in this kindred and the 20q13.3 region, thus providing evidence for locus heterogeneity for PHP-Ib. Sharing of only one parental haplotype by the affected individuals of this kindred excludes this chromosomal region also as a positional candidate for an autosomal recessive disorder. The loss of methylation at exon A/B observed in the affected individuals from this kindred, however, suggests a common pathway for the molecular mechanisms leading to the genetically distinct forms of PHP-Ib. Identification of the genetic mutation underlying the methylation defect in the kindred described above, and the actual mode of inheritance for this form of PHP-Ib will require further investigation and additional families with similar findings.

**Assembly and iron binding activity of human frataxin.** P. Cavadini, H. O'Neill, J. Adamec, G. Isaya. Departments of Pediatric and Adolescent Medicine and Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Rochester, MN.

Frataxin deficiency is the primary cause of Friedreich ataxia (FRDA), an autosomal recessive degenerative disease (Campuzano et al. 1996). Frataxin is a conserved mitochondrial protein that plays a critical role in cellular iron homeostasis. Increased oxidative damage and mitochondrial iron accumulation are associated with frataxin defects in yeast, mouse, and human cells (Babcock et al. 1997; Puccio et al. 2001; Wong et al. 1999) but the underlying biochemical defect is unknown. We have shown that ferrous iron stimulates self-assembly of yeast frataxin (Yfh1p) into regular multimers that store as many as 3,000 atoms of iron in soluble form (Adamec et al. 2000) and that this enables Yfh1p to stimulate heme synthesis and to prevent iron-induced Fenton chemistry *in vitro* (Adamec et al. submitted). Because human frataxin can maintain mitochondrial iron homeostasis in yeast (Cavadini et al. 2000), we have hypothesized that the yeast and human proteins share a similar mechanism of action. When expressed in *E. coli*, the mature form of human frataxin (residues 56-210) can be isolated as monomer as well as a high MW form (>600,000). Dynamic light scattering has shown that the high MW form has a hydrodynamic radius of  $14 \pm 2$  nm, and electron microscopy has revealed a spherical shape. This species is stable at 4°C but readily disassociates into monomer upon treatment with 0.25% SDS. As isolated from bacterial cells, the high MW form contains low levels of iron (<1 Fe/subunit) that can be increased to at least 10 Fe/subunit upon incubation with ferrous ammonium sulfate *in vivo*. The iron-loaded multimer can be separated on non-denaturing polyacrylamide gels and detected by protein or iron staining. These results suggest that, similar to Yfh1p, human frataxin is an iron-storage protein. The iron binding activity of frataxin may prevent iron-induced oxidative damage and mitochondrial iron accumulation *in vivo*.

This work is supported by grants from the MDA and the NIH/NIA (AG15709); P.C. is supported by a fellowship from the Mariani Foundation, Milan, Italy.

**Exclusion of two COX assembly genes in cytochrome c oxidase deficiency: SCO2 and COX10.** *S. LEBON, I. VALNOT, A. ROTIG, P. RUSTIN, A. MUNNICH.* INSERM U393, Hopital Necker, Paris, France.

Isolated cytochrome c oxidase (COX) is a frequent cause of respiratory chain defects in human and results in a variety of clinical manifestations including Leigh syndrome, cardiomyopathy, hepatic failure and encephalopathy. This clinical heterogeneity presumably stems from the large number of nuclear genes involved in the expression of the 13 COX subunit polypeptides and their subsequent maturation and assembly into the functional complex. In an attempt to better understand putative phenotype/genotype relationships, we screened 59 patients with isolated complex IV defects for mutations in two COX assembly genes previously shown to be involved in COX deficiency: SCO2 and COX10. SCO2 is involved in mitochondrial copper transport and/or insertion and mutations of this gene have been reported in several unrelated patients with cardioencephalomyopathy (Papadopoulou et al 1999, Nat Genet, 23, 333-7). On the other hand, COX10 encodes a heme A:farnesyl transferase and is mutated in COX-deficient patients presenting with proximal tubulopathy and encephalopathy (Valnot et al, 2000 Hum Mol Genet 9:1245-9). The coding sequences of the COX10 and SCO2 genes were tested by Denaturing High Performance Liquid Chromatography (DHPLC). Abnormal fragments were then directly sequenced. No mutations in SCO2 were identified in any patient (including those with cardioencephalomyopathy). Similarly, no mutations in COX10 could be detected either, confirming the very low incidence of COX10 mutations in patients with complex IV defects. Finally, we also studied COX11, COX15 and COX17 in few of these patients, but failed to identify any abnormality. This study brings support to the remarkable genetic heterogeneity of COX deficiencies in human.

**Using *Drosophila melanogaster* as a tool for analyzing human disease etiology.** E. Bier, L. Reiter. Cell and Developmental Biology, UCSD, La Jolla, CA.

We conducted an analysis of human disease gene homologues in *Drosophila melanogaster* with the goal of identifying genes amenable to study using *Drosophila* molecular genetics. Based on our analysis, which resulted in the identification of 714 human disease gene counterparts (see [Homophila](#)), we initiated studies into several human disease genes including those causing primary congenital glaucoma (PCG), Angelman syndrome (AS), and two antioxidant genes potentially involved in Alzheimer's disease (AD). We also began collaborations with human geneticists for each of these projects to integrate our studies into a gene discovery and analysis loop addressing the unanswered questions about various disease etiologies. The objective of our PCG study is to identify potential candidate genes in *Drosophila* corresponding to a suppressor locus on chromosome 8p that ameliorates the effects of *CYP1B1* mutations in some individuals. We generated a series of mutant alleles in a fly homolog of *CYP1B1* (*cyp18a*) which result in fluid flow problems reminiscent of those involved in PCG and are screening for second site suppressors of these mutations. Through a collaboration with Dr. B. Bejjani we will determine if any of these genes correspond to the suppressor locus he has mapped to human chromosome 8p by sequencing the human homologs of these genes in his patient pool. In AS the gene affected encodes an E3 ligase that targets certain proteins in the brain for degradation. To identify E3 ligase targets we will screen for suppressors of semi-lethal loss-of-function mutations in the *Drosophila* gene (*das*). In collaboration with Dr. A. Beaudet's group we will determine whether human or murine counterparts of these putative target proteins are present in elevated levels in a UBE3A<sup>-</sup> background. Finally, we will determine if the two antioxidant proteins we identified map to AD candidate loci through a collaboration with Dr. C. Van Broekhoven. Through these experimental approaches, we hope to validate *Drosophila* as a powerful model system for answering questions about disease gene function and set a precedent for establishing collaborations between clinical geneticists and researchers utilizing model organisms.

**A search for targets mediating Angelman syndrome pathogenesis using *Drosophila melanogaster*.** M. Bowers<sup>1</sup>, L.T. Reiter<sup>1</sup>, Y.-H. Jiang<sup>2</sup>, A.L. Beaudet<sup>2</sup>, E. Bier<sup>1</sup>. 1) Biology, UCSD, La Jolla, CA; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The human *UBE3A* gene encodes an E3 type ubiquitin ligase, E6-AP, whose function is to mark specific protein targets for ubiquitin-mediated degradation. *UBE3A* is expressed biallelically in most human tissues, but is genetically imprinted in the brain of humans and mice where only the maternal allele is expressed in the hippocampal and cerebellar neurons. Loss of function mutations in the maternal gene, paternal uniparental disomy, and imprinting mutations that prevent expression of both alleles result in Angelman syndrome (AS). Since it is thought that an overabundance of the proteins targeted by functional E6-AP is the cause of the neurological defects observed in AS patients, identification of these target proteins could lead to therapeutic strategies. Using the [Homophila](#) database, we identified a *Drosophila* homologue of *UBE3A*, which we named the *Drosophila* Angelman syndrome (*d-as*) gene. Utilizing an existing strain containing a *p*-element inserted into the 5'-UTR of *d-as*, we generated both lethal and semi-lethal alleles of *d-as*. The *p*-element line used also contains 14 copies of the yeast upstream activator sequence (UAS) which we used to misexpress *d-as* in various patterns via GAL4 expressing fly lines. Misexpression of *d-as* using the wing specific driver MS1096 results in the formation of ectopic wing veins and blisters indicative of a signaling pathway disruption. We also cloned the *d-as* cDNA and identified two spliceforms of the gene. The shorter transcript uses a cryptic splice site in exon V fused to exon I resulting in a much shorter protein product that is 71% similar to human *UBE3A*. In order to identify the protein targets of *d-as*, a genetic screen for second site suppressors of the *d-as* phenotype has been initiated. Once the *d-as* protein targets have been isolated in *Drosophila*, we will search for the corresponding proteins in mice and humans and assay their abundance in the brains of knock-out mice lacking *Ube3a*.

**The Jackson toxic milk mouse: a model for copper loading.** V. Coronado, M. Nanji, D.W. Cox. Department of Medical Genetics, University of Alberta, Edmonton, Alberta, Canada.

Copper is an essential trace element required for the function of several eukaryotic enzymes. However, when present in excess, copper becomes toxic, catalyzing the production of highly reactive oxygen species (ROS). Recently, ROS-induced oxidative stress has been implicated in a variety of neurological disorders, including Alzheimer disease (AD). Copper has been shown to accumulate in AD-affected brains and induce amyloid aggregation. Copper toxicity has been primarily studied in Wilson disease (WD), an autosomal recessive disorder in which ATP7B, a copper transporting ATPase expressed predominantly in the liver, is defective. Disruption of the copper transporting pathway results in decreased biliary excretion of copper and reduced incorporation of copper into ceruloplasmin. Consequently, copper accumulates in the liver, brain, and kidney, ultimately leading to liver cirrhosis and/or neurological damage frequently associated with kidney malfunction. Animal models of copper loading disorders are important in understanding these diseases and testing new potential therapies. The original toxic milk (*tx*) mouse, a WD model, has a Met1356Val substitution in the eighth transmembrane segment of Atp7b. In 1987, a new autosomal recessive mutation (*tx<sup>J</sup>*) arose in the C3H/HeJ mouse line (Jackson Laboratory) and has been maintained on the C3HeB/FeJ background, which is now a readily available *tx* strain. Like *tx/tx* mutants, homozygous *tx<sup>J</sup>* mice develop cirrhosis, and infant mice are pale colored, copper deficient, and display early mortality. The *tx<sup>J</sup>* mutation has been shown to be allelic with the original mutation, but the specific mutation has not been identified. We have cloned and sequenced the *tx<sup>J</sup>* Atp7b gene and conclude that the causative mutation is a Gly712Asp substitution in the second transmembrane segment of the deduced protein. This mutation creates a novel restriction site allowing for quick identification of normal and affected mice. Knowledge of this mutation will facilitate the use of this strain in studies of metabolic disorders and neurological diseases, in which copper accumulation plays an active role in pathology.

**An Animal Model of Mild Spinal Muscular Atrophy (SMA).** *U.R. Monani*<sup>1</sup>, *M. Pastore*<sup>2</sup>, *T. Gavrilina*<sup>1</sup>, *C. Andreassi*<sup>1</sup>, *A.H.M. Burghes*<sup>1,2</sup>. 1) Dept of Neurology, Ohio State Univ, Columbus, OH 43210; 2) Department of Molecular and Cellular Biochemistry, Ohio State Univ., Columbus OH 43210.

Spinal muscular atrophy (SMA), a neurodegenerative disorder, is the most common genetic cause of childhood death. It is caused by mutations in the *SMN1* gene and occurs in severe (type I), intermediate (type II) and mild (type III) forms. We have recently developed a mouse model of SMA. As in humans, mice lacking mouse *Smn* but carrying one or two copies of the human *SMN2* gene develop severe SMA and die by 8 days of age. What is more, *SMN2* can modify phenotypic severity and mice with 8 copies of the gene have a normal life-span and display no disease phenotype. Mice with 16 copies of *SMN2* are also normal indicating that over-expression of this protein is not deleterious. To generate a mouse with mild SMA, we have created a transgene with a missense mutation in exon 1 of the *SMN1* gene derived from a human patient. The transgene under the control of the SMN promoter was introduced onto the 1 or 2 copy *SMN2*; *Smn*<sup>-/-</sup> background. Preliminary results indicate that the *SMN2*; *Smn*<sup>-/-</sup> mice carrying the transgene display a typical type III SMA phenotype. They are smaller, display abnormal muscle and motor neuron pathology reminiscent of type III patients and are significantly weaker than their normal littermates. Further, although they develop normally, the mice die between 6 months of age and a year. These mice will be an important tool in testing drugs for the treatment of SMA, for which we and others have developed high-throughput screens.



**Functional conservation of the regulatory cascade for CFTR-mediated chloride secretion.** *J.E. Mickle<sup>1</sup>, K.J. Karnaky, Jr.<sup>2</sup>, G.R. Cutting<sup>1</sup>*. 1) Inst Genet Med, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Dept Cell Biol/Anatomy, Med Univ of South Carolina, Charleston, SC.

Cystic fibrosis (CF) is a life-limiting pulmonary disease that develops from abnormal ion transport. The gene responsible for CF encodes a protein, CFTR, that functions as an epithelial chloride channel and as a regulator of separate ion channels. The regulatory cascade of CFTR activation initiates when extracellular agonists bind b-adrenergic receptors; accordingly, proteins along the activation pathway are potential modifiers of the CF phenotype. However, the study of human CFTR in its native environment is hampered by low-level expression in relatively inaccessible tissues. Thus, a model system where native protein is highly expressed and readily available for functional analyses would be extremely beneficial. CFTR is abundantly expressed in chloride cells of killifish gill and opercular epithelium. The latter tissue is exceptional in that it is a naturally occurring polarized monolayer of epithelium suitable for functional genomic studies. To assess conservation of the CFTR regulatory pathway in killifish, opercular epithelium from seawater-adapted fish was mounted in Ussing chambers. After transepithelial voltage reached a stable value, the membrane was voltage-clamped and short circuit current (Isc) was recorded. No effect was observed upon the addition of 10mM isoproterenol, a b-adrenergic agonist, to the apical surface (Isc before:  $40.01 \pm 24.31$  SEM mAmp/cm<sup>2</sup>; Isc after:  $40.49 \pm 24.23$  mAmp/cm<sup>2</sup>,  $p = 0.42$ ,  $n = 3$ ). However, chloride transport was significantly increased by application of isoproterenol to the basolateral membrane (Isc before:  $37.16 \pm 10.88$  mAmp/cm<sup>2</sup>; Isc after:  $82.68 \pm 14.05$  mAmp/cm<sup>2</sup>,  $p = 0.004$ ,  $n = 7$ ). The response was inhibited by DPC and NBBP but not by DIDS. The functional measurements are consistent with molecular identification of CFTR in chloride cells; these measurements demonstrate that the regulatory cascade for CFTR activation is conserved in opercular epithelium. Thus, killifish provide a functional model similar to human epithelia that can be used to identify genes involved in the CFTR regulatory cascade using comparative genomic approaches.

**Characterization of the mouse orthologue of the human spastin gene to generate genetically engineered mouse models for autosomal dominant hereditary spastic paraplegia type 4 (SPG4).** *J. Schickel*<sup>1</sup>, *D. Boensch*<sup>1</sup>, *S. Klimpe*<sup>2</sup>, *R. Sudbrak*<sup>3</sup>, *G.E. Homanics*<sup>4</sup>, *T. Deufel*<sup>1</sup>. 1) Institut fuer Klinische Chemie und Laboratoriumsdiagnostik, FSU Jena, Jena, Germany; 2) Neurologische Universitaetsklinik, Mainz, Germany; 3) MPI fuer Molekulare Genetik, Berlin, Germany; 4) Department of Anesthesiology and Critical Care Medicine, University of Pittsburgh, Pittsburgh, USA.

Autosomal dominant hereditary spastic paraplegia (AD-HSP) is a genetically heterogeneous neurodegenerative disease characterised primarily by hyperreflexia and progressive spasticity of the lower limbs. The SPG4 locus at 2p21-p22 has been shown to account for approximately 40-50% of all AD-HSP families. The SPG4 gene was recently identified; it is ubiquitously expressed in adult and fetal tissues and encodes for spastin, an 616-amino-acid ATPase of unknown function belonging to AAA protein family. We have identified two novel SPG4 mutations in German AD-HSP families. A deletion of the first nucleotide of exon 9 leading to a premature stop codon is predicted to generate a truncated protein of 393 amino acids, and a large genomic deletion resulting in the loss of exons 13-16; patients carrying those mutations differ in their electrophysiological findings. With the aim to elucidate the function of the spastin gene and its role in the pathogenesis of spastic disorder, we have set out to generate genetically engineered mouse models carrying these two mutations defining distinct human disease phenotypes. As a first step we analysed the genomic organization of the mouse orthologue of SPG4 using screened BAC- and PAC-clones. The mouse gene consists of 17 exons spanning at least 35 kb of genomic DNA; subclones from the corresponding BACs/PACs were generated and provide the basis for the construction of targeting vectors to manipulate mouse embryonic stem cells and produce genetically altered mice.

**A mouse model for Smith-Magenis Syndrome.** *K. Walz, S. Carattini-Rivera, H. Vogel, R. Paylor, A. Bradley, J.R. Lupski.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Smith-Magenis Syndrome (SMS), associated with del(17)p11.2, is characterized by a genomic deletion of 4Mb. This deletion is believed to be the outcome of homologous recombination between flanking repeat gene clusters. The complex phenotypic features of SMS patients and the size of the deletion suggest that the haploinsufficiency of one or more genes in the commonly deleted region contribute to this disorder. Clinical features in humans include mental retardation, short stature, minor craniofacial anomalies, microcornea, developmental defects of the heart and kidneys, Smith-Magenis Syndrome (SMS), associated with del(17) and neurobehavioral abnormalities. The goal of this project is to generate a mouse model for SMS in order to define the gene or group of genes responsible for SMS phenotype. Human chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. Utilizing chromosomal engineering we deleted a region on mouse chromosome 11 that spans the syntenic region for the human commonly deleted interval. Mice heterozygous for the deletion in a 129SvEvBrd/C57BL/6 background were obtained at a mendelian rate. However, no homozygous mutant mice were recovered. Analysis of heterozygous mice indicated that some of the human clinical features are present in the model we generated. Craniofacial abnormalities were observed in the mutant mice with full penetrance. 20 percent of the mutant mice analyzed presented seizures, a percentage similar to the observed for human patients. No anatomical or histological anomalies were detected in a limited set of mutant mice analyzed (n of 6). Nevertheless, as the proportion of heart or kidney defects in the human SMS population is approximately 30 percent, more animals need to be studied. Behavioral studies are being done and analysis of the data is in progress.

**Transgenic expression of CECR1 results in developmental abnormalities similar to features of cat eye syndrome.**

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CECR1 is a novel putative growth factor/adenosine deaminase that maps within the critical region for cat eye syndrome encompassing approximately 2 Mb of DNA on chromosome 22q11. Common features of cat eye syndrome includes coloboma, preauricular pits and tags, heart (anomalous pulmonary venous defect, tetralogy of Fallot), and kidney (hypoplastic, absent) defects. CECR1 is expressed in a number of organs including heart and kidney and therefore is a candidate for features of cat eye syndrome. We have produced transgenic mice expressing CECR1 under the control of either a promoter with ubiquitous expression (beta-actin promoter) or a heart-specific promoter (alpha-myosin heavy chain promoter). Three beta-actin and three alpha-myosin heavy chain (MHC) lines with various integration sites and transgene copy numbers were analyzed in detail. The expression of CECR1 in the transgenic lines was confirmed with RT-PCR. The actin-CECR1 transgenic lines express CECR1 in most organs including the heart, kidney, and liver while the expression of CECR1 is mainly restricted to the heart in the MHC-CECR1 transgenic lines. A number of mice from each line were analyzed for phenotypic abnormalities. A high rate of embryonic and neonatal lethality was observed in two of the MHC-CECR1 lines. The transgenic mice produced with both constructs demonstrated cardiac hypertrophy. The hypertrophy was mostly due to the enlargement of the atria and right ventricle (both increased in weight by ~20%) as examined in 1-month old mice. In addition to the heart defects, the kidneys of the actin-CECR1 transgenics were hypoplastic or underdeveloped in two lines. Other abnormalities such as divided or cystic kidneys were occasionally observed in one line. Further study on the expression level of CECR1 in the transgenic mice as well as the specific cardiovascular defects in these mice is in progress. (Supported by the Lombard Insurance Chair in Pediatric Research and the Hospital for Sick Children Foundation).

**Triplet Duplication in  $\alpha 1(I)$  collagen chain of proband with lethal osteogenesis imperfecta shifts register of alpha chains in helix and alters incorporation of mutant trimers into fibrils and ECM.** W.A. Cabral<sup>1</sup>, M. Merts<sup>2</sup>, S. Leikin<sup>2</sup>, J.C. Marini<sup>1</sup>. 1) Section on Connective Tissue Disorders, HDB/NICHD/NIH, Bethesda, MD; 2) LPSB, NICHD/NIH, Bethesda, MD.

The helical region of type I collagen alpha chains consists of uninterrupted repeats of the amino acid triplet Gly-X-Y. The Gly-X-Y triplet defines the "register" of the alpha chains in collagen heterotrimer. We investigated the effect of shifting the interchain alignment of the collagen helix by a single Gly-X-Y triplet on collagen assembly, stability and incorporation into fibrils and matrix. These studies utilized a collagen mutation occurring in lethal type II OI. The proband has a triplet duplication in COL1A1 exon 44 cDNA and gDNA subclones. The normal allele encodes three Gly-Ala-Hyp triplets at aa 868-876, while the mutant allele encodes four. The register shift delays helix formation, causing overmodification of all CNBr peptides. Pericellular processing of secreted procollagen demonstrated more rapid appearance of pC- and mature collagen than control. In matrix deposited by proband cultured fibroblasts, overmodified chains were abundant in the immaturely cross-linked fraction but constituted a minor fraction of maturely cross-linked collagen. Trimers purified from cell culture exhibited markedly different behavior depending on whether they had two (+/+), one (+/-) or no (-/-) mutant  $\alpha 1$  chains. Differential scanning calorimetry revealed that +/+ molecules denature in sequential steps, 6°, 5° and 4°C below the T<sub>m</sub> of wild type protein; +/- molecules denature in a single step, 2°C below wild type. The three species appeared to be secreted in a 1:2:1 ratio consistent with random chain association. *In vitro* fibrillogenesis of this mixture produced fibers containing no +/+ molecules and a smaller +/- to -/- ratio than in the starting mixture. The rate and extent of fiber formation were comparable to wild type only at 4X higher protein concentration. Furthermore, turnover of +/- molecules was comparable to normal helices in ECM deposited by proband fibroblast cultures. The profound effects of shifting the collagen triplet register correlate with the severe clinical outcome.

Program Nr: 2672 from the 2001 ASHG Annual Meeting

**Molecular Basis of Mild form of Sickle cell -Beta Thalassemia syndrome in two Bahraini girls.** *S.S. Al Arrayed, N. Jassim.* Genetic Dept, Salmaniya Medical Ctr, Manama, Bahrain.

Two unrelated asymptomatic Bahrain female students were presented ,through a student screening project for hemoglobinopathies.with an atypical profile of HPLC of the whole blood .HPLC showed lower levels of HbA (20.8 and 21%).sickle Hb levels at 60.2and 62.4%.respectively.HbA2 levels at 8.9and7.3%.and elevated level of fetal hemoglobin at 7.9 and 6.8%.Both student were asymptomatic with normal clinical phenotype and without anemia.with HB of 12.7and 13.1% respectively.Molecular investigation by DGGE and RFLP studies revealed them to be compound heterozygotes for the sickle cell mutation (Bcd6A-T),and a B thal mutation nt-88(C-A)which is a mild B+ thalassemia mutation.Haplotype analysis indicates that sickle cell mutation is of the Saudi-Indian haplotype,while nt-88(C-A)mutation is associated with haplotype IX .Mapping of the alpha globin genes revealed both of the students as homozygotes for the rightward deletion(-a3.7/-a3.7).As a conclusion,these molecular investigation may explain unequivocally several of the bizzare clinical condions created by the presence of both sickle cell gene ,Beta thalassemia gene and alpha thalassemia gene in the same individual which is a common occurance in our region.

**Reduced hippocampal GABA<sub>A</sub> receptors and increased kainic acid seizure susceptibility in the fragile X mouse.**

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The Fragile X mental retardation syndrome often includes childhood seizures and electroencephalogram irregularities. Recent reports show that the Fragile X knockout (Fmr1 ko) mouse model for this disorder has increased susceptibility to audiogenic seizures. We find that these Fmr1 ko mice are also more sensitive to kainic acid (KA)-induced limbic seizures and show a striking increase in seizure-induced cell death in the CA3 subfield of the hippocampus. In vitro studies of cerebellar granule cells indicated that Fmr1 ko neurons are insensitive to inhibition by gamma-amino butyric acid (GABA). Immunohistochemical analysis of GABA<sub>A</sub> receptors in situ showed that these post-synaptic receptors were virtually eliminated from the Fmr1 ko mouse hippocampal formation. Hippocampal neurons normally express high levels of the Fmr1 gene product, FmrP, and GABA<sub>A</sub> receptor expression was partially rescued by introduction of a human FMR1 transgene. Taken together, these results indicate that the reduction of hippocampal GABA<sub>A</sub> receptors was due to the absence of FmrP in the Fmr1 ko mouse brain. Since expression of GABA<sub>A</sub> receptors depends in part on extracellular cues, the identity of the cell autonomously affected by the lack of FmrP is not yet clear. Nevertheless, reduced GABA<sub>A</sub> receptor expression in the hippocampal formation and the resulting loss of inhibition would explain the increased susceptibility to KA seizures and CA3 cell death in the Fmr1 ko mouse. GABA<sub>A</sub> receptor reduction was not apparent in the cerebellum or other brain areas, but it may be related to the increased audiogenic seizure susceptibility and altered startle response observed by others in the Fmr1 ko mouse. Decreased GABA inhibition may also contribute to the susceptibility to childhood seizures as well as some of the cognitive features in the Fragile X Syndrome.

**Procollagen from an OI proband with  $\alpha 1(I)$  exon 41 skipping has impaired binding of  $\alpha 1(I)$  C-telopeptide and confirms importance of this region for fibril assembly.** *J.C. Marini<sup>1</sup>, W.A. Cabral<sup>1</sup>, A. Fertala<sup>2</sup>, L.K. Green<sup>1</sup>, J. Korkko<sup>2</sup>, A. Forlino<sup>1</sup>.* 1) HDB/NICHD/NIH, Bethesda, MD; 2) Ctr for Gene Therapy, MCP Hahnemann University, Phila, PA.

Previous *in vitro* data on type I collagen self-assembly into fibrils suggested that the aa 776-797 region of  $\alpha 1(I)$  is crucial for fibril formation because it serves as the recognition site for the telopeptide of a docking collagen monomer. We used a natural collagen mutation with a deletion of aa 766-801 to confirm the importance of this region for collagen fibril formation. The proband is heterozygous for a COL1A1 IVS 41 A<sup>+4</sup>® C substitution. The intronic mutation causes splicing out of exon 41, confirmed by sequencing of normal and shorter RT-PCR products. The abnormal transcript is translated; structurally abnormal alpha chains are demonstrated in the cell layer of proband fibroblasts by SDS-Urea-PAGE. The proportion of mutant chains in the secreted procollagen was determined by digestion with MMP-1 to be 10%. Mutant chains are resistant to MMP-1 digestion because exon 41 also contains the vertebral collagenase cleavage site. The proportion of mutant protein was supported by quantitative RT-PCR on RNA from proband dermal fibroblasts and clonal cell lines, showing the mutant cDNA was about 15% of total  $\alpha 1(I)$  cDNA. Secreted proband collagen was used for analysis of kinetics of binding of  $\alpha 1C$ -telopeptide using an optical biosensor. Telopeptide had slower association and faster dissociation from proband than from normal collagen. Purified proband pC-collagen was used to study fibril formation. The presence of the mutant molecules decreases the rate of fibril formation. The fibrils formed in the presence of 10-15% mutant chain have an altered morphology, with increased length. These results suggest that trimers containing the mutant chain are incorporated into fibrils and that the absence of the telopeptide binding site from even a small portion of the monomers interferes with fibril growth. The severe type III OI phenotype of the proband may result from a combination of abnormal fibril morphology and longer persistence of mutant molecules in tissue, due to the lack of the MMP-1 cleavage site.



**Analysis of RNase MRP (RMRP) function in cartilage hair hypoplasia (CHH).** *P. Hermanns<sup>1</sup>, A. Bertuch<sup>1</sup>, A. Mian<sup>1</sup>, B. Zabel<sup>2</sup>, C. Bacino<sup>1</sup>, V. Lundblad<sup>1</sup>, B. Lee<sup>1</sup>.* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Children's Hospital of the University of Mainz, Germany.

Cartilage hair hypoplasia is an autosomal recessive disorder characterized by short stature, sparse hair, defective cellular immunity, and predisposition to several cancers. The critical region was mapped to 9p13 and recently, Ridanp et al. (2001) have shown that most CHH patients have mutations in the RNase MRP (RMRP) gene with one major Finnish allele in their population. RMRP is a ribonucleoprotein which functions as an endonuclease and is involved in processing of pre-rRNA in the nucleolus, cleavage of mitochondrial RNA, and priming of mitochondrial DNA replication. In a cohort of 15 patients we found mutations in both alleles in 13 patients. Two patients had no mutations in the RMRP gene. Of these, 21 mutations have not been previously identified. The mutations include insertions in the promoter, missense substitutions of evolutionarily conserved as well as divergent bases. The transcribed part of the RMRP gene is highly conserved among different species (mouse, rat, cow, human, xenopus, *A. thaliana*, *S. cerevisiae*). However, the only mutation, which is conserved between human and yeast is the main Finish mutation (70AG). This mutation has been reported to cause no reduction of RMRP level in patients or alteration of protein-RMRP binding. The RMRP complex still forms (Ridanp et al., 2001). To understand the function of RMRP mutations we have generated yeast targeted for the orthologous RMRP gene termed nuclear mitochondrial endonuclease 1 (NME1. The NME1 mutation causes cell lethality by tetrad analysis and current studies are aimed at rescuing the lethality with the human RMRP gene expressed from the endogenous promoter as well from a yeast ADH promoter. Human mutations will be introduced to ascertain their effects on cell viability, growth rate, growth phenotype at different temperatures, alteration in nutritional requirements, and changes in RNA and chromosomal stability including the ratio of 5.8SS and 5.8SL RNA. Together these studies will provide insight into the pathogenesis of the pleiotropic effects observed in CHH.

**Post mortem molecular genetic investigation of multiple hamartomas in a female TSC patient with pulmonary lymphangiomyomatosis.** *K. Mayer<sup>1</sup>, A. Dimmler<sup>2</sup>, A. Schaenzer<sup>2</sup>, H.D. Rott<sup>1</sup>.* 1) Institute of Human Genetics, Erlangen, Germany; 2) Institute of Pathology, Erlangen, Germany.

We report on the post mortem examination of a 41 year old female TSC patient who deceased from pulmonary lymphangiomyomatosis. Cerebral TSC manifestations were multiple cortical tubers with multiple astrocytic and multinuclear giant cells as well as a subependymal nodule with central calcification and statement of glial fibrillary acidic protein (GFAP). Renal involvement included four bigger renal angiomyolipomas up to 8x7 cm in size with central necrosis and multiple smaller tumours within the renal cortex. Cause of death was a chronic right heart insufficiency originated by pulmonary lymphangiomyomatosis. Native samples from multiple brain and kidney hamartomas as well as from normal tissue were obtained for molecular genetic analyses. In order to identify the combination of both inactivating TSC mutations in different lesions we started with LOH screening in both TSC genes using microsatellite markers analyzed with Genotyper 2.5 on an ABI 310 sequencer and single nucleotide polymorphisms analyzed with DHPLC which offers the possibility of a quantitative determination of allele ratios in tumour versus normal tissue. We continued with exon screening applying DHPLC and direct sequencing if no LOH was detected. Fibroblast cultures obtained from pericard and hamstring were screened to confirm the patients germline mutation. The results of this study will offer the opportunity to give insights to the molecular pathogenesis of individual hamartomas in the same patient according to the two hit tumour suppressor inactivation model.

**Peripheral nervous system involvement in laminin alpha2 chain-null mutant ( $dy^{3K}/dy^{3K}$ ) mice.** *M. Nakagawa*<sup>1,3</sup>, *Y. Miyagoe-Suzuki*<sup>2</sup>, *K. Ikezoe*<sup>3</sup>, *Y. Miyata*<sup>4</sup>, *I. Nonaka*<sup>3</sup>, *K. Harii*<sup>5</sup>, *S. Takeda*<sup>2</sup>. 1) Plastic and Reconstructive Surgery, Saitama Medical School, Saitama, Japan; 2) Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; 3) Ultrastructural Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan; 4) Pharmacology, Nippon Medical School, Tokyo, Japan; 5) Plastic, Reconstructive and Aesthetic Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.

The laminin alpha2 chain is major component of basal lamina in both skeletal muscle and the peripheral nervous system. The laminin alpha2 chain deficiency causes merosin-deficient congenital muscular dystrophy, which affects not only skeletal muscle, but also the peripheral nervous system. It has been reported that the formation of basal lamina is required for myelination in the peripheral nervous system. In fact, the spinal root of dystrophic mice ( $dy/dy$  mice), whose laminin alpha2 chain expression is greatly reduced, shows lack of basal lamina and amyelination. To investigate the role of laminin alpha2 chain and basal lamina in vivo, we examined the peripheral nervous system of  $dy^{3K}/dy^{3K}$  mice, which are null mutants of laminin alpha2 chain. The results indicate the presence of myelination although Schwann cells lacked basal lamina in spinal root of  $dy^{3K}/dy^{3K}$  mice, suggesting that basal lamina is not an absolute requirement for myelination in vivo. Immunohistochemically, the expression of laminin alpha4 chain was increased and laminin alpha5 chain was preserved in endoneurium of the spinal root. Laminin alpha4 and alpha5 chains may play the critical role in myelination instead of laminin alpha2 chain in  $dy^{3K}/dy^{3K}$  mice. In addition, the motor conduction velocity of the sciatic nerve was significantly reduced. This reduction in conduction velocity may be due to small axon diameter, thin myelin sheath and patchy disruption of the basal lamina of the node of Ranvier in  $dy^{3K}/dy^{3K}$  mice.

**The Townes-Brocks syndrome gene SALL1 is a transcriptional repressor and interacts with UBC9 and SUMO-1.**  
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The SALL1 gene encodes a putative zinc finger transcription factor (1324 aa) which is mutated in Townes-Brocks syndrome. This syndrome is inherited as an autosomal dominant trait and is characterized by imperforate anus, preaxial polydactyly, and dysplastic ears. Little is known about the cellular function of SALL1. To investigate its possible role as a transcription factor we performed reporter gene assays expressing portions of SALL1 fused to the GAL4 DNA binding domain transiently in mammalian cells. These experiments revealed that the central 1000 aa of SALL1 strongly repress the expression of a luciferase reporter gene controlled by the thymidine kinase promoter and GAL4 DNA binding sites. Trichostatin A, an inhibitor of histone deacetylases, was not able to relieve this repression suggesting that SALL1-mediated repression does not lead to the recruitment of mSin3A, N-CoR or histone deacetylases. We also employed the yeast two-hybrid system to identify protein interaction partners of SALL1. One of these interacting proteins is UBC9 which is known to mediate the covalent modification of other proteins by SUMO-1. The SALL1/UBC9 interaction was confirmed in vitro by a GST pulldown experiment. We could also demonstrate interaction between SALL1 and SUMO-1 in the yeast system indicating that SALL1 is modified by sumoylation. Sumoylation is known to target proteins to certain subnuclear compartments. Expression of a GFP-SALL1 fusion protein in NIH3T3 cells showed indeed localization of GFP-SALL1 in distinct nuclear speckles.

**The CCM1 gene product KRIT1 interacts with the integrin binding protein ICAP-1.** *J.S. Zawistowski<sup>1</sup>, I.G. Serebriiskii<sup>2</sup>, M.F. Lee<sup>1</sup>, E.A. Golemis<sup>2</sup>, D.A. Marchuk<sup>1</sup>.* 1) Department of Genetics, Duke University Medical Center, Durham, NC; 2) Division of Basic Science, Fox Chase Cancer Center, Philadelphia, PA.

Cerebral Cavernous Malformations (CCM) are vascular anomalies of the brain consisting of grossly enlarged capillary-like vessels with no intervening neural tissue. Individuals affected with CCM most often present with the symptoms of seizures, intracerebral hemorrhage leading to stroke and focal neurological deficits. Our lab and others have identified the CCM1 disease gene as *Krit1*, a protein initially identified based on a yeast two-hybrid interaction with the Ras-family GTPase RAP1A. As the function of the KRIT1 protein and its role in CCM1 pathogenesis remain unknown, we performed yeast two-hybrid screens to identify additional protein binding partners. Utilizing the Dual Bait two-hybrid system (Serebriiskii et al. 1999 *J. Biol. Chem.* 274(24):17080), we used the N-terminal 276 amino acid residues of KRIT1 as bait, a region lacking similarity to any known protein upon database searches. Much of this region has been recently identified as additional upstream coding sequence not originally reported as part of the *Krit1* coding region (Sahoo et al. 2001 *Genomics* 71(1):123). From parallel screens of human fetal brain and HeLa cDNA libraries, we obtained multiple independent isolates of human ICAP-1 (integrin cytoplasmic domain-associated protein-1) as interacting clones. The specificity of the N-terminal KRIT1/ICAP-1 interaction within the context of the yeast two-hybrid system was verified by retransformation assays performed in parallel with a non-specific bait. The  $\alpha$  isoform of ICAP-1 is a serine/threonine-rich phosphoprotein which binds the cytoplasmic tail of  $\beta_1$  integrins. In addition, its phosphorylation status is regulated during cell-matrix interactions, suggestive of the protein playing an important role in integrin-mediated cell adhesion. The interaction between ICAP-1 and KRIT1, and the presence of a FERM domain in the latter, suggest that KRIT1 may be involved in the bidirectional signaling between integrin molecules and the cytoskeleton. Furthermore, these data suggest that CCM1 pathogenesis might involve cell adhesion processes via integrin signaling.

**Impaired *IMPT1* expression in kidney stone disease demonstrated by reverse transcription-polymerase chain reaction *in situ* hybridization.** A. Sahota<sup>1</sup>, E.G. Tzortzaki<sup>1</sup>, M. Yang<sup>1</sup>, D. Glass<sup>1</sup>, J. Barrera-Oro<sup>1</sup>, L. Deng<sup>1</sup>, A.P. Evan<sup>2</sup>, S. Bledsoe<sup>2</sup>, P.J. Stambrook<sup>3</sup>, J.A. Tischfield<sup>1</sup>. 1) Dept. Genetics, Rutgers Univ., Piscataway, NJ; 2) Dept. Anatomy and Cell Biology, Indiana Univ. Sch. Med., Indianapolis, IN; 3) Dept. Cell Biology, Neurobiology, and Anatomy, Univ. Cincinnati Coll. Med., Cincinnati, OH.

The mRNA for imprinted multimembrane-spanning polyspecific transporter-like gene 1 (*IMPT1*) is highly expressed in organs with metabolite transport functions, including the kidney. In a knockout mouse model for adenine phosphoribosyltransferase (APRT) deficiency and 2,8-dihydroxyadenine (DHA) nephrolithiasis, we previously observed a decrease in *IMPT1* mRNA expression in the kidney, suggesting that this may be responsible, at least in part, for the impaired renal function in these mice. To assess the functional significance of this observation, we identified the cell types expressing *IMPT1* mRNA in paraffin-embedded mouse kidney sections. *IMPT1* mRNA was not detected using *in situ* hybridization (ISH), but we observed intense nuclear staining in the cortex, medulla, and glomeruli from wild-type mice using an improved RT-PCR ISH procedure. We used only six PCR cycles, and uniform heat distribution over the slides, in order to minimize tissue damage and/or PCR product diffusion during amplification. *IMPT1* expression was significantly decreased in all three regions in kidney sections from APRT-deficient male mice, and this was confirmed by solution RT-PCR. APRT-deficient female mice are less severely affected by DHA lithiasis than their male counterparts, and we observed only a modest reduction in *IMPT1* expression in these mice. *IMPT1* expression in *Aprt* heterozygotes was comparable to that in wild-type mice, suggesting imprinting of one of the parental alleles. These findings suggest that one consequence of renal deposition of DHA in APRT deficiency is impaired *IMPT1* mRNA expression and this may have adverse effects on glomerular and tubular transport functions. RT-PCR ISH can be used to localize the site of expression of transcripts that may not be detectable using standard ISH procedures. Supported by NIH grants DK38185, ES05652, and ES06096.

**DNA rearrangements at the D4Z4 locus in South African facioscapulohumeral muscular dystrophy families.** A. van der Merwe<sup>1</sup>, C-M. Schutte<sup>1</sup>, S.M. van der Maarel<sup>2</sup>, M. Alessandrini<sup>1</sup>, E. Honey<sup>1</sup>, R.R. Frants<sup>2</sup>, A. Olckers<sup>1</sup>. 1) University of Pretoria, South Africa; 2) Leiden University Medical Centre, The Netherlands.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant, late-onset degenerative neuromuscular disorder. Disease onset occurs generally within the first or second decade of life. The phenotypical expression varies in the age of onset and clinical severity. FSHD is characterised at onset by the progressive weakness and atrophy of the facial and shoulder girdle muscles. Linkage was established to the sub-telomeric region of chromosome 4q35. This sub-telomeric region contains a polymorphic repeat array locus (D4Z4) consisting of 3.3 kb repeats. An integral number of repeats are deleted in affected individuals. Integrated haplotypes were constructed for 177 individuals from five extended South African FSHD families. Southern Blot analysis was performed to determine DNA rearrangements in 162 individuals from this selected group of families. Deletion fragments were observed in all families investigated. A BlnI resistant deletion fragment of 24 kb segregated with the postulated FSHD-haplotype in families F10, F30 and F40. The FSHD-haplotype in all three families was identical, with the entire nine-allele haplotype segregating in one sub-family of F10 (F15) and in family F30. Six alleles of this haplotype co-segregated with the FSHD phenotype in F13 (sub-family of F10) and three alleles co-segregated in F40. It was previously reported that an identical six allele FSHD-haplotype segregated in families F20 and F60. The deletion fragments segregating in these families were also of identical size. To date, only two FSHD haplotypes have been observed in the South African population - each co-segregating with a deletion fragment of a specific size.

**The Dystrophin-Associated Complex in *Drosophila* and Lamprey.** *M.J. Greener*<sup>1</sup>, *G. Tear*<sup>2</sup>, *R.G. Roberts*<sup>1</sup>. 1) Div. of Med. & Mol. Genet., Guy's Hospital, London, UK; 2) MRC Centre for Developmental Neurobiology, Guy's Hospital, London, UK.

Mutations in the genes for dystrophin, and other members of the dystrophin-associated complex (DAC) result in phenotypically related forms of muscular dystrophy. Work in mammals is somewhat complicated by the diversity of proteins which contribute to the DAC in different tissues and subcellular localizations (3 dystrophin-like proteins, 2 dystrobrevins, 5 syntrophins, 5 sarcoglycans).

We have found that the model invertebrate *Drosophila melanogaster* has a substantially simpler repertoire of orthologous proteins (1 dystrophin, 1 dystrobrevin, 2 syntrophins, 3 sarcoglycans). In an attempt to assess whether fruitfly dystrophin performs a single ancestral role or functionally encompasses the fundamental activities of all three human dystrophin-like proteins (dystrophin, utrophin, DRP2), we have performed in-situ hybridization and stage-specific expression analysis. These suggest both a neuronal and muscular function, consistent with a "union set" of vertebrate dystrophin roles.

To provide an evolutionary "snapshot" of a situation intermediate between invertebrates and mammals, we examined the agnathon fish, lamprey. Amplification of reverse transcripts using degenerate oligonucleotides revealed the presence of two dystrophin-like sequences. Phylogenetic analysis suggests that one of these is a common orthologue of dystrophin and utrophin, while the other is more DRP2-like. This is consistent with the 2R hypothesis, whereby much of the chordate genome was duplicated in two events, one before and one after the divergence of lampreys.

This work will help to complete our picture of the DAC as a biological entity which has diversified with the growing complexity of the host organism, and will help to broaden our understanding of the etiology of muscular dystrophy.



**Temporal expression profiling in a progressive disease, Duchenne muscular dystrophy.** *Y.-W. Chen<sup>1</sup>, M. Bakay<sup>1</sup>, R. Borup<sup>1</sup>, S. Toppo<sup>2</sup>, G. Valle<sup>2</sup>, G. Lanfranchi<sup>2</sup>, E.P. Hoffman<sup>1</sup>.* 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) CRIBI Biotechnology Center, University of Padua, Padua, Italy.

Duchenne muscular dystrophy (DMD) is a progressive muscular disorder caused by mutations in the dystrophin gene. Although the primary biochemical defect is known, the downstream biochemical changes leading to later patient weakness and death are not understood. To identify differentially expressed genes involved in the progression of the disease, we report expression profiling of pre-symptomatic, early-stage and mid- to end-stage DMD muscles by using custom made Affymetrix MuscleChip containing approximately 1000 full-length genes and 2000 muscle ESTs. Among the total 4,654 probe sets on the MuscleChip, expression of 3688 (80%) genes was detected by the MuscleChip at least once out of 46 chips in this study. We found a large number of significant gene expression changes in fetal muscles (193 up- and 8 down-regulated genes) years before the onset of symptoms. By applying both correlation and K-means analysis, 48 genes were identified up-regulated during the progression of the DMD while down-regulated in the age-matched control individuals. One hundred and fifty seven genes were down-regulated during disease progression while up-regulated in control individuals. Among the down-regulated genes, 55% of the known genes were mitochondrial and metabolic genes. Genes involved in regulation of oxidative stress, protein metabolism were also identified. The major up-regulated genes are genes involved in fibrosis and extracellular matrix remodeling, muscle fiber structure, protein synthesis and degradation. Interestingly, expression levels of many genes that we previously reported misregulated by comparing pooled 5-9-year-old DMD vs. control (ex.  $\alpha$ -cardiac actin and chondroitin sulfate proteoglycan versican) showed correlation to the progression of the disease. By comparing the pre-symptomatic, early- and mid- to late-stage DMD profiles, we set the stage for progression-specific therapeutic targets for this common and lethal inherited disease.

**Deficiency of a-dystrobrevin and b2-syntrophin defines a new form of inherited myopathy.** *K.N. North<sup>1</sup>, K.J. Jones<sup>1</sup>, A. Compton<sup>1</sup>, M.M. Mills<sup>1</sup>, N. Yang<sup>1</sup>, D. Mowat<sup>2</sup>, M. Peters<sup>4</sup>, L.M. Kunkel<sup>3</sup>, S.C. Froehner<sup>4</sup>.* 1) Institute for Neuromuscular Research, Childrens Hospital at Westmead, Sydney, Australia; 2) Sydney Children's Hospital, Australia; 3) Children's Hospital, Boston, MA; 4) University of Washington, Seattle, WA.

The syntrophins and dystrobrevins are members of the dystrophin-associated-protein complex expressed at the sarcolemmal membrane and at the neuromuscular junction (NMJ). They are excellent candidate genes for human muscle disease based on their reduced expression in primary dystrophinopathy. In addition, both a1-syntrophin and a-dystrobrevin knockout mice demonstrate muscle pathology. We have characterized the expression of the syntrophins and a-dystrobrevin in normal human muscle and in biopsies from 162 patients with myopathies of unknown etiology. All patient biopsies had normal staining with antibodies to dystrophin, the sarcoglycans, sarcospan, caveolin, laminin-a2, a- and b1-syntrophin. Deficiency of a-dystrobrevin, in some cases accompanied by loss of b2-syntrophin, was found in 16/162 (10%) patients. All 16 patients presented with congenital-onset hypotonia and weakness. Two major clinical patterns emerged: patients with deficiency of b2-syntrophin and a-dystrobrevin presented in the newborn period with severe skeletal and respiratory muscle weakness and died in the first year of life. CK was normal. One infant from a consanguineous family had a sibling and first cousin with the same clinical picture and identical immunocytochemical profile, suggestive of AR inheritance. The remaining cases were sporadic. Two patients with deficiency of a-dystrobrevin alone had congenital muscular dystrophy and complete external ophthalmoplegia. One of these demonstrated clinical improvement on acetylcholinesterase inhibitors, suggesting that NMJ dysfunction was contributing to his clinical picture. We have sequenced a-dystrobrevin and b2-syntrophin but have not identified any mutations. This suggests that disease-causing mutations occur outside the coding region of these genes or in a gene encoding another component of the syntrophin-dystrobrevin subcomplex. We are currently screening other dystrobrevin binding proteins.

**Pathological and genetic analysis of the *degenerating muscle (dmu)* mouse: a new allele of *Scn8a*.** R. Kothary<sup>1,2,3</sup>, P.D. Côté<sup>1,2</sup>, M. Pool<sup>1,2,3</sup>, S. Girard<sup>3</sup>, S.M. Vidal<sup>3</sup>, Y. De Repentigny<sup>1,2</sup>. 1) Ottawa Health Research Institute, Ottawa, ON, Canada; 2) University of Ottawa Center for Neuromuscular Disease; 3) University of Ottawa, Ottawa, ON, Canada.

Mouse mutants suffering from muscle defects have served as valuable models in the identification of genes implicated in human neuromuscular disorders as well as in the elucidation of the pathogenesis of muscle disease. Here, we describe a novel spontaneous autosomal recessive mutation in the mouse that is characterized by skeletal and cardiac muscle degeneration. We have named this mutant *degenerating muscle (dmu)*. At birth, mutant mice are indistinguishable from their normal littermates. Thereafter, the disease progresses rapidly and a phenotype is first observed at approximately 11 days after birth; the *dmu* mice are weak and have great difficulty in moving. The principal cause of the lack of mobility is muscle atrophy and wasting in the hindquarters. Affected mice die at or around the time of weaning of unknown causes. Histopathological observations and ultrastructural analysis revealed muscle degeneration in both skeletal and cardiac muscle, but no abnormalities in sciatic nerves. Using linkage analysis, we have mapped the *dmu* locus to the distal portion of mouse chromosome 15 in a region syntenic to human chromosome 12q13. Interestingly, scapuloperoneal muscular dystrophy (SPMD) in humans has been linked to this region. SPMD patients with associated cardiomyopathy have also been described in the past. Initial analysis of candidate genes on mouse chromosome 15 reveal that although intact transcripts for *Scn8a* are present in *dmu* mice, their levels are dramatically reduced. Furthermore, genetic complementation experiments between *dmu* and *med* (mutation in *Scn8a*) mice reveal that they are allelic, although their phenotypes are not entirely identical. Our results suggest that at least a portion of the *dmu* phenotype is caused by a down-regulation of *Scn8a*, the gene encoding the sodium channel 8a subunit.

*Supported by : Canadian Institutes of Health Research and the Muscular Dystrophy Association (USA).*

**Syncoilin Expression in Neuromuscular Disorders.** *E.V. Howman<sup>1</sup>, E. Poon<sup>1</sup>, S.E. Newey<sup>1</sup>, S. Torelli<sup>2</sup>, S. Brown<sup>2</sup>, D. Hilton-Jones<sup>3</sup>, W. Squier<sup>3</sup>, F. Muntoni<sup>2</sup>, K.E. Davies<sup>1</sup>.* 1) MRC Functional Genetics Unit, Department of Human Anatomy and Genetics, Oxford University, Oxford, United Kingdom; 2) The Dubowitz Neuromuscular Centre, Department of Paediatrics, ICSM, London, United Kingdom; 3) Department of Neurobiology, Radcliffe Infirmary, Oxford, United Kingdom.

We recently identified a new member of the dystrophin-associated protein complex, syncoilin, through its interaction with  $\alpha$ -dystrobrevin in skeletal muscle (Newey et al, 2001). Syncoilin is found at the sarcolemma and the neuromuscular junction, where it colocalizes with  $\alpha$ -dystrobrevin, and is also found at the Z-lines of skeletal muscle. Syncoilin is proposed to be a member of the intermediate filament superfamily based on sequence analysis. Based on the cellular localization, we hypothesize that syncoilin may form a link between the dystrophin-associated protein complex and the intermediate filament network found at the sarcomere. We have shown that syncoilin is upregulated in pathological conditions suggesting that it may have a role in maintaining the integrity of muscle fibres through the stabilization of the intermediate filament network. The critical role of intermediate filaments in muscle has been highlighted by the identification of mutations in the genes for several intermediate filaments in various myopathies associated with aberrant intermediate filament organisation. In this study we have assessed syncoilin expression in a range of neuromuscular disorders including Duchenne muscular dystrophy, Becker muscular dystrophy, congenital muscular dystrophy, spinal muscular atrophy and desmin-related myopathies. The aim was to a) develop an understanding of the role syncoilin plays in the stabilization of the muscle fibre and b) to screen patients with a myopathy of unknown causation because a dysfunction or absence of syncoilin may result in the disruption of the intermediate filament network leading to muscle necrosis. The results of these case studies will be presented. Newey et al. (2001). Syncoilin, a novel member of the intermediate filament superfamily that interacts with  $\alpha$ -dystrobrevin in skeletal muscle. *J. Biol. Chem.* 276, 6645-6655.

**A novel RYR1 mutation Q474H identified in a Japanese family with malignant hyperthermia and central core disease.** *K. Ogata*<sup>1,2</sup>, *J. Goto*<sup>1,2</sup>, *S-Y. Jeong*<sup>1,2</sup>, *Y. Takahashi*<sup>1,2</sup>, *J. Shimizu*<sup>1</sup>, *M. Yoshimura*<sup>1</sup>, *H. Kikuchi*<sup>3</sup>, *Y. Ichihara*<sup>3</sup>, *I. Kanazawa*<sup>1,2</sup>. 1) Dept Neurol, Grad Sch Med, Univ Tokyo, Tokyo, JAPAN; 2) SORST, JST, Kawaguchi, Saitama, JAPAN; 3) First Dept Anesthesiol, Toho Univ Sch Med, Tokyo, JAPAN.

Malignant hyperthermia (MH) in humans is an autosomal dominant skeletal muscle disorder characterized by hypercatabolic reactions induced by anesthetic agents, with symptoms of sustained uncontrolled skeletal muscle calcium regulation resulting in life-threatening organ and systemic failure. Central core disease (CCD) is an autosomal dominant congenital myopathy characterized by hypotonia and muscle weakness with the 'central core' microscopical change in muscle fibers. Genetic investigations revealed that missense mutations in the ryanodine receptor 1 gene (*RYR1*) can cause both MH susceptibility (MHS) and/or CCD. MHS with RYR1 mutation is named MHS1. Here we report a novel missense mutation of RYR1 mutation in a Japanese family with MH and CCD.

Four affected members of this family showed mild muscle weakness and elevated serum creatine kinase levels. One member had an episode of MH while under general anesthesia. CT images demonstrated diffuse mild fatty changes in skeletal muscles of two affected members. Biopsied muscle from the proband of this family showed mild myopathic changes with type 1 predominancy and cores in muscle fibers. Functional tests with saponized muscle fibers demonstrated increase in calcium-induced calcium release rate. Direct sequencing and denaturing high-performance liquid chromatography analysis with the genomic PCR product from three affected and two unaffected members of this family revealed Q474H missense mutation in RYR1. All affected patients were heterozygous for the mutation. Sixty unrelated subjects, taken as controls did not demonstrate a similar mutation. This novel mutation is located in one of the main region for pathogenic mutation, the so-called foot region of RYR1. Our result is supportive of the hypothesis that this region is essential for the regulation of calcium release from sarcoplasmic reticulum through RYR1.

**Hereditary rippling muscle disease is caused by caveolin-3 mutations.** C. Kubisch<sup>1</sup>, R. Betz<sup>1</sup>, B. Schoser<sup>2</sup>, D. Kasper<sup>2</sup>, K. Ricker<sup>3</sup>, A. Ramirez<sup>1</sup>, V. Stein<sup>2</sup>, T. Torbergesen<sup>4</sup>, Y.-A. Lee<sup>5</sup>, M. Noethen<sup>1</sup>, T. Wienker<sup>6</sup>, J.-P. Malin<sup>7</sup>, P. Propping<sup>1</sup>, A. Reis<sup>5</sup>, W. Mortier<sup>8</sup>, T. Jentsch<sup>2</sup>, M. Vorgerd<sup>7</sup>. 1) Institute of Human Genetics, University of Bonn, Germany; 2) Center of Molecular Neurobiology, University of Hamburg, Germany; 3) Department of Neurology, University of Würzburg, Germany; 4) Department of Neurology, University of Tromso, Norway; 5) Gene Mapping Center, University of Berlin, Germany; 6) IMBIE, University of Bonn, Germany; 7) Department of Neurology, University of Bochum, Germany; 8) Department of Pediatrics, University of Bochum, Germany.

Rippling muscle disease (RMD; MIM 600332) is an autosomal dominant disorder characterized by mechanically-triggered, electrically silent contractions of the skeletal muscle. The genetic cause of this non-dystrophic muscle disease is unknown. By genome-wide linkage analysis we found evidence for linkage in three unrelated German RMD families on human chromosome 3p25. Testing of the positional candidate gene *caveolin-3* (*CAV3*) identified missense mutations in those three and additional two RMD-families including the original Norwegian RMD family first described in 1975. Some of these mutations have already been described in limb-girdle muscular dystrophy type 1C (LGMD1C) or idiopathic elevation of serum creatine kinase. All mutations lead to a reduced cav3 surface expression in muscle biopsies or stably transfected C2C12 myotubes, while the localization of a variety of calcium transport proteins of the skeletal muscle and sarcolemmal neuronal nitric oxide synthase (nNOS) was unaltered. Interestingly, NO-production in response to cytokine stimulation was 30-40% higher in cells transfected with mutant cav3 than in controls. In summary, the mislocalization of cav3 and the associated increased inducibility of nNOS may contribute to the mechanically induced muscle contractions in RMD. Moreover, the allelism between dystrophic LGMD1C and non-dystrophic RMD suggests that hereditary caveolinopathies may represent a valuable model to identify factors which determine whether a muscle disease shows a non-dystrophic or a dystrophic course.

**Sarcomeric proteins and Neuromuscular disorders.** *M. Vainzof*<sup>1</sup>, *G. Faulkner*<sup>2</sup>, *G. Valle*<sup>3</sup>, *A. Beggs*<sup>4</sup>, *O. Carpen*<sup>5</sup>, *P. Salmikangas*<sup>5</sup>, *C. Wallgren-Pettersson*<sup>5</sup>, *S. Labeit*<sup>6</sup>, *J. Gurgel-Giannetti*<sup>7</sup>, *E.S. Moreira*<sup>1</sup>, *M.R. Passos-Bueno*<sup>1</sup>, *M. Zatz*<sup>1</sup>.  
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The sarcomere is the unit of skeletal and cardiac muscle contraction. In the past few years, there has been major efforts in the attempt to elucidate the role of skeletal and cardiac muscle proteins. Mutations in several sarcomeric proteins such as actin, nebulin, alpha-tropomyosin 3, TPM-2 and Troponin T1 have been found to cause nemaline myopathy, while mutations in the genes for telethonin and myotilin, autosomal recessive LGMD-2G and autosomal dominant LGMD-1A, respectively. The number of known protein components of the sarcomere is rising, and new proteins, such as ZASP, FATZ (myozenin or Calsarcin), and Ankrd2, have recently been identified. We have been analyzing the status of proteins from the sarcomere in a large cohort of patients affected by the several forms of dystrophies and myopathies in the Brazilian population. To date, mutations in the nebulin gene have been identified in one family and a deficiency of the C-terminal domain of nebulin was observed in a second NM patient. Screening for myotilin deficiency in 20 candidate patients showed normal results, while deficiency of telethonin/T-cap was detected in 4 out of 153 LGMD families. Normal expression of the newly described ZASP, FATZ and Ankrd2 sarcomeric proteins was observed by immunohistochemical analyses, in studied 20 muscle biopsies from patients with clinical and histopathological characteristics of MD, previously excluded for the other known muscle protein deficiencies (dystrophin, the 4 sarcoglycans, calpain, dysferlin). The study of sarcomeric proteins is important for understanding the physiopathology of neuromuscular diseases and to establish genotype/phenotype correlation at the protein level. Financial support: CEPID-FAPESP, PRONEX, CNPq, ABDIM.

**Genotype-phenotype correlation in nemaline myopathy.** *M.M. Ryan<sup>1</sup>, C. Schnell<sup>2</sup>, C.D. Strickland<sup>1</sup>, L.K. Shield<sup>3</sup>, G. Morgan<sup>4</sup>, S.T. Iannaccone<sup>2</sup>, N.G. Laing<sup>5</sup>, K.N. North<sup>6</sup>, A.H. Beggs<sup>1</sup>.* 1) Neurology and Genetics, Children's Hospital, Boston, MA; 2) Neurology, Texas Scottish Rite Hospital, Dallas, TX; 3) Neurology, Royal Children's Hospital, Victoria, Australia; 4) Genetics, Sydney Children's Hospital, Sydney, Australia; 5) Australian Neuromuscular Research Inst., Nedlands, Australia; 6) Neurogenetics, Children's Hospital at Westmead, Sydney, Australia.

Nemaline myopathy (NM) is a clinically heterogenous disorder defined by the presence of rods or nemaline bodies in muscle fibres. Disease-causing mutations have been identified in 5 genes, all encoding components of the muscle thin filament. We have characterized the clinical, pathological and genetic features of 118 cases of NM. Inheritance was autosomal recessive in 26 patients from 16 kindreds and autosomal dominant in 27 patients from 22 kindreds. 65 cases were sporadic. There was marked intrafamilial phenotypic heterogeneity within both autosomal dominant and recessive kindreds. Disease severity was not predictive of mode of inheritance. 16/59 patients tested had mutations in the skeletal muscle  $\alpha$ -actin gene (*ACTA1*). Clinical presentation and course varied widely in these cases. One patient with severe neonatal weakness was a compound heterozygote for two *ACTA1* mutations. 12 patients were sporadic cases with new dominant mutations in *ACTA1*. Three of 35 patients tested had mutations in  $\alpha$ -tropomyosin<sub>SLOW</sub> (*TPM3*).

On light microscopy, rod number and localisation did not correlate with disease severity or mode of inheritance. Simultaneous biopsies of different muscles revealed variable disease expression. Serial biopsies showed progressive fibre size variation, fibrosis and an increase in the number of rods over time. Fibre hypertrophy was associated with a milder phenotype. Considerable pathologic heterogeneity was seen where multiple family members were biopsied. Tropomyosin NM was characterised by marked variation in fibre size and preferential rod formation in type 1 fibres. Glycogen accumulation was prominent in actin NM, and the degree of sarcomeric disruption on electron microscopy correlated with disease severity.



**Gene expression in nemaline myopathy skeletal muscle.** *D. Sanoudou<sup>1</sup>, J.N. Haslett<sup>1</sup>, S. Greenberg<sup>2</sup>, H.G.W. Lidov<sup>1</sup>, I.S. Kohane<sup>2</sup>, L.M. Kunkel<sup>1</sup>, A.H. Beggs<sup>1</sup>.* 1) Genetics Div., Childrens Hosp, Boston, MA; 2) Bioinformatics Program, Children's Hospital, Boston, MA.

The nemaline myopathies (NM) are a clinically and genetically heterogeneous group of disorders characterized by nemaline rods and primary skeletal muscle weakness that is usually congenital. They vary from rapidly fatal in the neonatal period to relatively nonprogressive and compatible with normal life span and activity. Five different NM genes (ACTA1, NEB, TPM2, TPM3, TNNT1) have been identified, however, the molecular consequences of these mutations are unknown. Using Affymetrix oligonucleotide microarrays we have analyzed the expression patterns of over 12,000 genes in the skeletal muscles of 12 NM patients. The datasets obtained were compared to a baseline of 10 normal skeletal muscle specimens and datasets from other congenital myopathies and dystrophies. Conventional fold-difference comparisons, as well as automated classification and clustering techniques were used to identify genes with significant differences in their expression patterns between NM and normal samples. Of the 100 genes with the strongest hybridization signals, 28 were ribosomal, and 23 were muscle specific. 41 ribosomal genes were significantly overexpressed compared to all normal muscle samples. This feature was unique for NM patients, and was not seen in DMD or other diseases we studied. This provides evidence to suggest that protein turnover may be increased, despite the non-degenerative appearance of NM muscle. A number of genes encoding sarcomeric proteins were also overexpressed (troponin T1, troponin C,  $\alpha$ -actin, filamin, nebulin and myomesin). All of these genes showed normal expression in the other muscle diseases studied. Overall, the gene expression patterns in NM patients exhibit a distinct clustering pattern, reflecting uniqueness in the molecular pathophysiology of this disorder. Some of the changes identified are expected to be disease- and/or gene-specific while others will reflect non-specific responses of myopathic muscle. Knowledge of these alterations will shed light on both the unique functions of NM genes as well as on more general aspects of skeletal muscle pathophysiology.

**Novel Dysferlin Mutations in Brazilian LGMD2B Patients.** *F. Paula<sup>1</sup>, M. Vainzof<sup>1</sup>, E.S. Moreira<sup>1</sup>, M.R. Passos-Bueno<sup>1</sup>, K. Bushby<sup>2</sup>, R. Bashir<sup>2</sup>, M. Zatz<sup>1</sup>.* 1) Ctr Study Human Genome, Univ de São Paulo, São Paulo, SP, Brazil; 2) Dept of Biochemistry and Genetics, Newcastle Upon Tyne, Newcastle Univ, UK.

The limb girdle muscular dystrophy 2B (LGMD2B) and Miyoshi myopathy (MM) are caused by allelic mutations in the dysferlin gene. Only few pathogenic changes have been reported to date. In order to establish a genotype-phenotype correlation we are analyzing the distribution of dysferlin mutations in patients from 18 LGMD2B and MM Brazilian families, classified by linkage analysis (10) or dysferlin deficiency (8).

Through SSCP, dHPLC and sequencing of abnormal fragments we identified to date 5 different mutations in 7 unrelated families: 2 missense changes in heterozygosity (the L189V in 2 families and D396H in one, exons 6 and 13 respectively), one stop codon in exon 28 (W999X) in 2 families (one homozygous and another in heterozygous state) and 2 frameshift mutations in the last 2 families: 3522-3523delTC (in one allele) and 3446-3453insCAGTGCTT (a duplication of 8pb in homozygosity state), both in exon 29. Dysferlin protein analyzed in muscle from all of these families showed absence of this protein. No hot spot for mutations was identified.

All these patients related weakness first in the distal muscles of the lower limbs and involvement in upper limbs on average 10 years later, but with a variable course even in patients belonging to the same family. All showed slow progression and on average, the phenotype in patients with nonsense mutations was not more severe than among those with missense mutations. In one of the families with the W999X mutation, the parents (deceased in their sixties) who were first degree cousins, were both affected. They had 10 children, also with a variable course. The intrafamilial variability, despite the high degree of consanguinity in this unusual family gives further support to the existence of modifying factors modulating the severity of the phenotype. Supported by FAPESP/CEPID, CNPq, PRONEX and IAEA.

**Expression profiling in facioscapulohumeral muscular dystrophy (FSHD) indicates a defect in myogenic differentiation.** *S. Winokur<sup>1</sup>, J. Martin<sup>1</sup>, Y-W. Chen<sup>2</sup>, J. Ehmsen<sup>1</sup>, K. Flanigan<sup>3</sup>.* 1) Dept Biological Chemistry, Univ California, Irvine, CA; 2) Children's National Medical Center, Washington, D.C; 3) Eccles Institute of Genetics, University of Utah, Salt Lake City, UT.

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder resulting from integral deletions of a 3.3 kb tandem repeat (D4Z4) in the subtelomeric region of chromosome 4q. Although the specific genes affected by this deletion have not been identified, a regional disruption of chromatin structure affecting local gene expression is thought underlie the pathophysiology. The global gene expression profiling of mature muscle tissue in FSHD presented here elucidates aberrant cellular processes previously concealed by this unusual molecular mechanism. FSHD expression profiles were compared to those generated from normal muscle and other types of muscular dystrophies (DMD, aSGD, JDM) in order to determine FSHD specific changes. In addition, matched biopsies (affected and unaffected muscle) from patients with FSHD served to monitor expression changes during the progression of the disease and to reduce non-specific changes resulting from individual variability. Among 15 genes upregulated in an FSHD specific and highly significant manner were several involved in cellular differentiation and proliferation. These include muscle LIM protein (MLP), delta homolog (DLK1) and the mitotic inhibitor huWEE1. As MLP and DLK were not increased further in the affected to unaffected FSHD muscle, these may represent a primary change in FSHD rather than an effect of progressing dystrophy. Increased MLP and DLK expression was confirmed by both real time RT-PCR and a second GeneChip format. The majority of FSHD-specific genes with reduced expression reflect a diminished capacity to buffer oxidative stress. These FSHD specific downregulated genes include SNAPC1 (HHCPA78 homolog) and nuclear factor of kappa light polypeptide gene alpha (NFKB1A). Enhanced vulnerability to oxidative stress is a characteristic previously demonstrated in cultured FSHD undifferentiated myoblasts. We propose a model linking aberrant myogenic differentiation and oxidative stress to subtelomeric shortening in FSHD.

**Developmental and overexpression studies of CASK in skeletal muscle.** *J.L. Siders<sup>1</sup>, T.A. Hainsey<sup>1</sup>, S. Murnaghan<sup>2</sup>, J.B. Wilson<sup>2</sup>, J.A. Rafael<sup>1</sup>.* 1) Department of Molecular and Cellular Biochemistry, College of Medicine, The Ohio State University, Columbus, OH; 2) I.B.L.S. Division of Molecular Genetics, University of Glasgow, 54 Dumbarton Rd., Glasgow, U.K.

The role of PDZ domain-containing proteins in skeletal muscle has yet to be clearly defined. We have previously shown that CASK and Dlg, two PDZ domain-containing proteins, are present in skeletal muscle and specifically at the post-synaptic membrane of the neuromuscular junction. CASK and Dlg belong to the membrane associated guanylate kinase family of proteins and are known to cluster channels and receptors at central nervous system synapses. These PDZ domain-containing proteins are therefore likely to play a significant role at the skeletal muscle neuromuscular junction and in neuromuscular diseases. We have conducted studies using the C2C12 mouse myogenic cell line to further characterize the role of these proteins in skeletal muscle. These studies demonstrate that CASK is expressed in a developmental specific manner. CASK localizes to the nucleus in cultured myoblasts, then is transported out to the cytoplasm as cells differentiate and fuse into multinucleated myofibers. As these fibers mature CASK protein expression greatly increases. Dlg shows only a slight increase in expression as myofibers mature. Interestingly, neither of these proteins cluster in response to agrin-induced acetylcholine receptor clustering suggesting a novel mechanism of localization of CASK and Dlg to the neuromuscular junction. This myogenic culture system will be used for transfection experiments to determine critical protein interaction domains of CASK. Transgenic mouse lines overexpressing full length CASK around the entire skeletal muscle membrane have been generated. Analysis of these mice and comparisons with mice overexpressing a PDZ domain truncated version of CASK will further elucidate the role of these proteins in vivo in skeletal muscle and in neuromuscular diseases.

**Two novel mutations in the glycine decarboxylase (P-protein) gene in patients with nonketotic hyperglycinemia (NKH).** *D.A. Applegarth*<sup>1,2,3</sup>, *J.R. Toone*<sup>1,3</sup>, *M.B. Coulter-Mackie*<sup>1,2</sup>. 1) Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada; 2) Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; 3) Biochemical Diseases Laboratory, B.C.'s Children's Hospital, 4480 Oak Street, Vancouver, British Columbia, V6H 3V4, Canada.

A defect in the P-protein gene causes NKH in more than 80% of patients. The molecular study of this gene has been hampered by the presence of a processed full-length pseudo-gene and few mutations have been identified. Two recurring mutations have been reported: S564I in Finnish patients and R515S (now found in 8 unrelated cases).

We have characterised the region of exons 6 and 7 and intervening sequence (GenBank AF288639) which represents >10% of the coding region of the P-protein gene and contains sequences of the gene conserved from human to *E.coli*. Using 1 PCR product and 1 sequencing primer, we have tested 10 patients shown to have a P- or H-protein defect by liver glycine exchange assay. Three had already been identified as heterozygous for R515S.

We found 3 positive alleles: one patient was homozygous for P329T, a conserved amino acid in a conserved region of the gene in exon 7; a second patient was heterozygous for A283P in exon 6. A283P is not completely conserved but a PCR/SacI restriction digest assay showed that it was absent in 100 normal alleles. The introduction of a proline residue is expected to disrupt secondary protein structure.

Many NKH patients investigated in Japan (Kure et al (1999) *Am J Hum Gen* 65 (4), abstract 2406) have been shown to have rare or private mutations. Identifying regions of clusters of mutations will aid in a customised approach for sequencing DNA of patients who do not have a recurrent mutation.

This work was supported by a grant from the Garrod Association of Canada.

**Detection of Mutations in the Dystrophin Gene Via Automated DHPLC Screening and Direct Sequencing.** *R.R.*

*Bennett*<sup>1</sup>, *J. denDunnen*<sup>2</sup>, *K. O'Brien*<sup>3</sup>, *B. Darras*<sup>1</sup>, *L. Kunkel*<sup>1,3,4</sup>. 1) Division of Genetics, Children's Hospital, Boston, MA; 2) Department of Human and Clinical Genetics, Leiden University Medical Center, Leiden, Netherlands; 3) Department of Genetics, Harvard Medical School, Boston, MA; 4) Howard Hughes Medical Institute, Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA.

Duchenne Muscular Dystrophy (DMD) is a genetic disorder which manifests itself in one of every 1600 to 2000 live births. One in 3200 to 4000 will be severely disabled boys and one in 3200 to 4000 will be carrier females with reproductive complications and potential late-onset cardiac complications. Approximately 65% of mutations causing this disease are large deletions or duplications, most of which are found by the existing set of multiplex PCR primers. The other 35% have remained undetected in most patients. The purpose of this project was to develop an efficient and inexpensive process for detecting mutations large and small. We have obtained sequence upstream and downstream of each exon as well as the 5' and 3' UTRs from the NIH human genome database and have designed primers to include 30 to 100 bases on either side of each exon. These primers were designed to produce a single clean band on agarose gel, to create DNA fragments with melting characteristics appropriate for DHPLC analysis using the WAVE from Transgenomic Inc. for DNA variation screening, and finally to sequence well on automated DNA sequencers. We have tested eight patients --selected from medical records as clearly having DMD but no large mutation-- using these new primers in conjunction with the WAVE and PE-ABI 373 sequencer. We have detected six disease-causing mutations. The remaining two patients have been completely sequenced. Several polymorphisms have been detected in each, most of which are known to be non disease-causing. Further investigation is required to determine the cause of DMD in these two patients.

**Identification of non-pathogenic mutations by a new statistical approach. Analysis of CFTR gene mutations in a random population.** *F. Belpinati*<sup>1</sup>, *S. Giorgi*<sup>2</sup>, *M. des Georges*<sup>3</sup>, *V. Scotet*<sup>4</sup>, *M. Toepfer*<sup>5</sup>, *C. Bombieri*<sup>1</sup>, *M. Fagioli*<sup>2</sup>, *A. Bouchut*<sup>3</sup>, *M.P. Audrezet*<sup>4</sup>, *A. Begnini*<sup>1</sup>, *E. Guidotti*<sup>2</sup>, *B.M. Ciminelli*<sup>2</sup>, *M. Macek jr*<sup>5</sup>, *C. Ferec*<sup>4</sup>, *M. Claustres*<sup>3</sup>, *G. Modiano*<sup>2</sup>, *P.F. Pignatti*<sup>1</sup>. 1) Section of Biology & Genetics, DMIBG, Univ. of Verona, Italy; 2) Dpt. Biol. E.Calef, Univ. Roma Tor Vergata, Italy; 3) Inst. Biol., Univ. Montpellier, France; 4) Centre Biogenet. CDTS, Brest, France; 5) Dpt. Mol. Genet., Charles Univ., Prague, Czech Rep.

It may be difficult to determine the role of not obviously pathogenic mutations in causing disease, in particular when a high number of mutations is known. We have proposed a straightforward method to classify non pathogenic mutations. Given  $q$  as the global frequency of all the alleles causing a disease, any allele with a frequency certainly higher than  $q$  minus the cumulative frequency of the already known disease causing mutations cannot be the cause of that disease. This principle was applied to the analysis of CFTR gene mutations as cause of CF. In the first part of the work, the CFTR gene (coding and proximal non coding region) of 191 DNA samples from random individuals from Italy, France and Spain was investigated. A total of 46 mutations was detected: 3 were known to be CF-causing and 13 were classified as non CF-causing alleles (Hum Genet 2000;106:172). We here present the second part of the work. The analysis of the 30 mutations not yet classified was extended by DGGE and restriction testing to other 1265 individuals (2530 genes) from Italy, France, and the Czech Republic. This is sufficient to classify mutations with a frequency of at least 0.01. A total of 41 mutations, 11 of which were found during the second step, was detected: 8 mutations (R75Q, 1716G/A, G576A, R668C, 3041-71G/C, 4404C/T, 3030G/A, 1001+11C/T) were classified as non CF-causing, 2 were not described mutations (A1009T, 3813A/G), 3 mutations were known to be CF-causing. In conclusion, on a total of 57 mutations identified in the two steps of the work: 21 mutations (37%) were classified as non CF-causing on the basis of their frequency, 6 were known to be CF-causing and 6 new mutations were first described.

**MEFV and TNFRSF1A gene mutations in patients suffering from AA amyloidosis.** *C. Dode<sup>1</sup>, C. Pecheux<sup>1</sup>, D. Cattan<sup>2</sup>, B. Hazenberg<sup>3</sup>, B. Moulin<sup>4</sup>, A. Barthelemy<sup>5</sup>, M-C. Gubler<sup>6</sup>, M. Delpech<sup>1</sup>, G. Grateau<sup>7</sup>.* 1) EMI 00-05, INSERM, Paris, France; 2) Service d'hépatogastroentérologie, Centre hospitalier, Villeneuve-St Georges, France; 3) Rheumatology Department, Academic Medical Hospital, Groningen, The Netherlands; 4) Service de néphrologie et d'hémodialyse, Hôpital civil Strasbourg, France; 5) Service de néphropédiatrie, Hôpital Necker, Paris, France; 6) INSERM Unité 423, Hôpital Necker Paris, France; 7) Service de médecine interne, Hôtel-Dieu, Paris, France.

Mutation analysis in MEFV and TNFRSF1A genes was carried out in 40 patients suffering from AA amyloidosis associated with recurrent inflammatory attacks. MEFV mutations were only found in patients belonging to FMF at risk ethnic origins (34 patients) such as Sephardic Jews, Turks, Armenians, Arabs from the Maghreb. 22/34 patients displayed an homozygous MEFV mutation (19 M694V/M694V, 2 M694I/M694I, 1 M680I/M680I). 2/34 patients had a single mutations (M694V/M694V) and 10/34 had no mutation. Among patients with a single one or without mutation, we searched for mutations in TNFRSF1A gene. Five novel mutations were found: C55S, C70Y, R92Q, C96Y in Caucasian patients and the Y20H mutation was found in a Sephardic patient. In this series, FMF was the main cause of AA amyloidosis in Sephardic Jewish and Turkish populations. In the Maghrebian patients, AA amyloidosis was due to MEFV mutations in only 5/14 cases suggesting another cause for renal AA amyloidosis. In two cases, this cause seemed to be due to an autosomal recessive disease of unknown origin. Our results showed that TRAPS is not always found in Caucasian populations but also in patients belonging to at-risk FMF ethnies such as Sephardic Jews. The molecular diagnosis is very important because the therapeutic behaviors are different for these two diseases.



**An APC allele harbouring both Y159X and E1317Q in a large family with attenuated familial adenomatous polyposis.** *N. Al-Tassan<sup>1</sup>, J. Maynard<sup>1</sup>, N. Fleming<sup>1</sup>, D.R. Davies<sup>2</sup>, J.R. Sampson<sup>1</sup>, J.P. Cheadle<sup>1</sup>.* 1) Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, UK; 2) Dept. of Medicine, University Hospital of Wales, Cardiff, UK.

It has been proposed that seemingly benign missense variants in the APC gene show association with colorectal adenoma and carcinoma. The variant, E1317Q, has been found to be over represented in the constitutional DNA of patients with multiple colorectal adenomas and carcinoma and has been identified as a somatic change in sporadic colorectal cancer. We have investigated a large family (17 members with multiple adenomas and / or carcinoma) with an attenuated FAP (AFAP) phenotype. Sequence analysis of the APC coding region identified the missense variant E1317Q in all affected family members, but also the earlier exon 4 nonsense mutation Y159X. This early truncating mutation has been previously reported in another AFAP family. We suggest that the Y159X mutation is likely to be the cause of colorectal adenoma and carcinoma predisposition in the present family, although a contributory affect of the E1317Q allele through low level alternate splicing cannot be formally excluded at present.

**Identification of a putative mosaic *MECP2* mutation in an atypical Rett syndrome patient by denaturing high performance liquid chromatography.** *K.R. Bowles<sup>1</sup>, I.M. Buysse<sup>1</sup>, K.T. Hoon<sup>1</sup>, M.T. Bashford<sup>1</sup>, P. Arn<sup>2</sup>, B.B. Roa<sup>1</sup>.* 1) Baylor DNA Diagnostic Laboratory, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Division of Genetics, Nemours Children's Clinic, Jacksonville, FL.

Rett syndrome is an X-linked dominant neurodevelopmental disorder wherein affected females appear normal at birth, followed by deterioration of higher brain function, loss of motor and language skills, acquired microcephaly, ataxia, seizures, and autistic behavior beginning at 6-18 months of age. The Rett syndrome gene *MECP2* encodes methyl-CpG-binding protein 2. Clinical diagnostic testing for *MECP2* mutations is performed in our laboratory using denaturing high performance liquid chromatography (DHPLC) and automated DNA sequence analysis. We studied an atypical Rett syndrome patient who presented at 2 years of age, but retained purposeful hand movements, is not microcephalic, and has a normal MRI. Analysis of patient DNA by DHPLC showed a consistently abnormal chromatogram indicative of a sequence variant; however, DNA sequencing of the corresponding PCR product indicated a wild-type sequence. The specific PCR product was subsequently fraction-collected off the DHPLC column, and individual fractions were used as templates for PCR and sequence analysis. Sequencing of fractions that coincided with the variant DHPLC peak identified a single base change, 1093 G-T, predicting a nonsense mutation, E365X. The low-level mutation in this patient was further confirmed by Pyrosequencing, an automated primer extension-based assay, which estimated an allele ratio of 82:18 (normal:mutant). Similar results were obtained with DNA samples from patient peripheral blood lymphocytes, buccal cells, and cultured fibroblasts. Analysis of maternal DNA did not identify the E365X nonsense mutation, suggesting a *de novo* *MECP2* mutation in the patient. In conclusion, a putative mosaic *MECP2* nonsense mutation was identified by three different methods in a patient with atypical Rett syndrome. Under optimized conditions, DHPLC analysis may provide greater sensitivity than DNA sequence analysis for the detection of certain low-abundance mutations.

**Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma.** *D. Astuti<sup>1</sup>, F. Latif<sup>1</sup>, N. Morgan<sup>1</sup>, T. Martinsson<sup>2</sup>, A. Dallol<sup>1</sup>, P. Dahia<sup>3</sup>, F. Douglas<sup>4</sup>, E. George<sup>5</sup>, F. Sköldbberg<sup>6</sup>, E. Husebye<sup>6</sup>, C. Eng<sup>7</sup>, E.R. Maher<sup>1</sup>.* 1) Section of Medical and Molecular Genetics, University of Birmingham, Birmingham B15 2TT, UK; 2) Department of Clinical Genetics, Gothenburg University, Gothenburg, Sweden; 3) Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02115, USA; 4) Northern Regional Genetics Service, Royal Victoria Infirmary, Newcastle upon Tyne NE2, UK; 5) Department of Medicine, Kings Lynn Hospital, Norfolk, UK; 6) Dept. of Medical Sciences, Uppsala University, Sweden; 7) Clinical Cancer Genetics and Human Cancer Genetics Programs, Comprehensive Cancer Center, and the Division of Human Genetics, The Ohio State University, Columbus, OH 43210, USA.

Pheochromocytoma susceptibility may be associated with germline mutations in the VHL and NF1 tumour suppressor genes and the RET proto-oncogene. However, the genetic basis for most cases of nonsyndromic familial pheochromocytoma is unknown. Recently pheochromocytoma susceptibility was associated with germline SDHD gene mutations which were originally described in hereditary paraganglioma. The SDHD and SDHC gene products anchor the two other components of the mitochondrial complex II (SDHA and SDHB), which form the catalytic core, to the mitochondrial inner membrane. Although mutations in SDHD and SDHC may cause hereditary paraganglioma, germline mutations in SDHA are associated with juvenile encephalopathy and the phenotype of SDHB mutations had not been defined. To investigate the genetic causes of pheochromocytoma we analysed the SDHB and SDHC genes in familial and sporadic cases. Inactivating SDHB mutations were detected in 2/5 kindreds with familial pheochromocytoma, 2/3 kindreds with pheochromocytoma and paraganglioma susceptibility and 1/24 cases of sporadic pheochromocytoma. These findings suggest that germline SDHB mutations are an important cause of pheochromocytoma susceptibility. As SDHB maps to 1p36, a region deleted in many tumor types we have proceeded to analyse sporadic neuroblastomas, lung tumours and renal carcinomas for somatic SDHB mutations.

**MAPH detection of deletions/duplications in Duchenne/Becker Muscular Dystrophy: an alternative to quantitative Southern blotting.** *M.H. Breuning, S. White, M. Kalf, M. Villerius, E. Bakker, G.-J.B. van Ommen, J.T. den Dunnen.* Center for Human and Clinical Genetics, LUMC, Leiden, Netherlands.

Currently most genetic diagnostic protocols are PCR-based and do not readily yield quantitative data. As a consequence, potential deletions and duplications of the regions analyzed go undetected unless specific methods are applied. Southern blotting is most commonly used, but is time consuming and laborious. Recently an alternative method was published, called Multiplex Amplifiable Probe Hybridization (MAPH). In this technique a series of short DNA fragments are cloned in such a way that all can be PCR-amplified using one pair of primers. The probes are hybridized to genomic DNA immobilized on nylon filters, and after stringent washing the probes are recovered off the filters and PCR-amplified in a quantitative manner. We have applied this technique to the diagnosis of Duchenne/Becker Muscular Dystrophy (DMD/BMD), diseases caused by mutations in the dystrophin gene. This gene is the largest known, covering 2.4 Mb, containing 79 exons. In approximately 65 % of cases the mutation is a deletion or duplication of one or more exons. Frame-shift mutations cause the lethal DMD, whereas maintenance of the reading frame leads to the less severe BMD. For this reason it is important to assess the boundaries of rearrangements, a potentially arduous task. A technique allowing simultaneous analysis of all exons would greatly simplify this procedure. We cloned all 79 exons into the same vector and divided the PCR products into 2 pools. Following hybridization the secondary PCR was performed using a fluorescently labeled primer, allowing the products to be analyzed on a 96 capillary sequencer. This allows parallel analysis of 96 samples in ~48 hours. The number of copies of each exon could be determined by comparing the appropriate peaks between controls and patients. Using this technique we were able to detect exon deletions and duplications missed using current methods. Further probe sets are being developed to cover other areas of interest such as breast cancer and deletion syndromes. The possibility of analyzing many more regions simultaneously using microarrays is being investigated.

**ND gene: Mutations found in two Portuguese families.** *H.F.R. Caria<sup>1</sup>, M. Vitorino<sup>1</sup>, A. Mena<sup>1</sup>, I. Galhardo<sup>2</sup>, M. Simao<sup>2</sup>, O. Dias<sup>2</sup>, M. Andrea<sup>2</sup>, C. Correia<sup>1</sup>, G. Fialho<sup>1</sup>.* 1) Center of Genetics and Molecular Biology, University of Lisbon, Portugal; 2) Center of CORL, University of Lisbon, Portugal.

Norrie disease (ND, McKusick n 31060) is a rare and a severe X-linked form of congenital blindness accompanied by mental retardation and deafness in at least one third of the patients. The blindness is bilateral and caused by a vascularized mass behind each lens due to a maldeveloped retina (pseudoglioma). The ND gene spans 28 kb of genomic DNA comprised in three exons where the coding portion includes the half part of the exon 2 and the first part of exon 3. The ND gene encodes a 133 aminoacids protein, which function is not well known, although homologies with known proteins and molecular modelling data suggest a role in the regulation of cell interaction or differentiation processes. In the present work two unrelated portuguese families with Norrie disease were analysed. In one family the screening of the entire coding region allowed the identification of a IVS2-1G>C mutation in all the affected males. For the same region, the other family exhibits a single base pair transversion (G>C) at nt828, apparently not related to the disease. The mutations were detected by sequencing analysis and confirmed by restriction analysis. DNA analysis was also used to determine the carrier status of females.

**Mutations in the COL4A4 and COL4A3 Genes Cause Familial Benign Hematuria.** *C. Badenas<sup>1</sup>, B. Tazon<sup>1</sup>, L. Heidet<sup>3</sup>, C. Arrondel<sup>3</sup>, M. Mila<sup>1</sup>, C. Antignac<sup>3</sup>, A. Darnell<sup>2</sup>, R. Torra<sup>2</sup>.* 1) Genetics, Hospital Clinic, Barcelona, Barcelona, Spain; 2) Nefrologia, Hospital Clinic, Barcelona, Barcelona, Spain; 3) Nephrology Department. INSERM U423, Universit Ren Descartes, Hpital Necker-Enfants Malades, Paris, France.

Familial benign hematuria is a common autosomal dominant disorder characterised by the presence of persistent or recurrent hematuria. The clinical and pathological features of this syndrome resemble those of early Alport syndrome and for this reason a common molecular defect has been proposed. The COL4A3/4 genes seem to be involved in both autosomal Alport syndrome and familial benign hematuria. We have performed linkage analysis for the COL4A3/4 loci and searched for mutations within these genes in 11 biopsy proven FBH families. The linkage studies demonstrated that six out of 11 families were linked to the COL4A3/4 locus, and one was not linked. However this family included three affected women who could be carriers of an X-linked Alport syndrome. Four families were too small to perform linkage analysis, but in two of them linkage to the COL4A3/4 was possible. The mutational studies disclosed two new mutations in the COL4A3 gene (G985V, G1015E) and four new mutations in the COL4A4 gene (3222insA, IVS23-1G>C, 31del11, G960R) It is the first time that mutations within the COL4A3 gene are described in families with FBH. The present study clearly demonstrates the involvement of the COL4A4 and COL4A3 genes in the pathogenesis of FBH.

**High frequency of autosomal recessive forms of Chronic Granulomatous Disease in Tunisian patients.** R. EL Kares, M.R. Barbouche, S. Abdelhak, K. Dellagi. Laboratoire d'Immunologie, Institut Pasteur de Tunis, Tunisia.

Chronic granulomatous disease (CGD) is a group of inherited disorders of host immune system caused by genetic defects in any of the four components of phagocyte NADPH oxidase, namely gp91-, p22-, p47-, and p67-phox. This deficiency leads to a profound impairment of microbicidal functions of the phagocytic system. The most common form as far reported is X-linked and is due to defects in the *CYBB* gene that encodes for gp91-phox. The remaining cases are inherited in an autosomal recessive mode, through mutations in the genes for p22- (*CYBA*), p47- (*NCF-1*) or p67-phox (*NCF-2*). Mutations in the gp91-, p22- and p67-phox are highly heterogeneous. In contrast a single defect corresponding to a GT deletion (DGT) in exon 2 of *NCF-1* gene accounts for the vast majority of p47-phox deficiency cases. In the present study a total of 13 CGD patients (7 males and 6 females) have been investigated. The diagnosis of CGD was confirmed on the basis of the absence of nitroblue tetrazolium (NBT) reduction by the patient neutrophils. Most of these patients (75%) are born to consanguineous parents. In this context of high consanguinity the strategy used to identify genes involved is homozygosity mapping using microsatellites markers within the region of *CYBA*, *NCF-1* and *NCF-2* genes mapped respectively to 16q24, 7q11.23 and 1q25. Linkage to chromosome 16, 7 and 1 has been found for 4, 5 and 1 patients respectively. Three patients show no homozygosity with any of the autosomal loci. Since these patients are males, X-linked gene mutation could not be ruled out. The study of mutations within the corresponding genes were investigated by RT-PCR amplification and direct sequencing. Along with recurrent mutations that have been identified in other studies such as the recurrent (DGT) deletion, we report a novel mutation in the *CYBA* gene corresponding to a deletion of 7 base-pairs in exon 5 designated as 295 del 7. Our results show the higher frequency of linkage to autosomal recessive genes in Tunisian patients affected with CGD compared to data from USA, Europe and Japan. This is probably the consequence of the higher rate of consanguinity in our population.

**GSD type II: Description of four novel mutations causing acid  $\alpha$ -glucosidase deficiency.** *D. Bali, A.J. McVie-Wylie, M. Lowery, E. Faulkner, D. Lamson, Y.T. Chen.* Div. of Medical Genetics, DUMC, Durham, NC, 27710.

Glycogen storage disease type II (GSD II, Pompe disease) is a lysosomal storage disease resulting from deficiency of the enzyme acid  $\alpha$ -glucosidase (GAA). Clinically, Pompe disease encompasses a range of phenotypes from a severe, fatal infantile form characterized by cardiomyopathy and skeletal muscle weakness to a slowly progressive adult presentation limited to skeletal muscle. Over 50 mutations causing GSD-II have been described; here we reported four novel mutations in two Caucasian patients with the severe infantile presentation of the disease and less than 1% normal GAA activity. Patient one is a compound heterozygote with a maternally inherited 2bp frameshift deletion in exon 4 (721-722 delTT) and a paternally inherited nonsense mutation (C1687T, Gln563Ter) introducing a stop codon in exon 12. Western blot analysis indicates that this patient does not make detectable protein and cDNA analysis was impossible due to a lack of amplifiable material. Therefore, it is probable that both of these mutations result in unstable mRNA production and consequently no protein production. Patient two is also a compound heterozygote with a paternally inherited mutation in the intron 9 splice acceptor site resulting in the deletion of exon 10 (IVS9(-1G>C)). Since part of the catalytic site of GAA is present within exon 10, we assume that exon 10 skipping would result in a non-functional protein. The maternally inherited mutation in this patient results in a missense change in the protein within exon 12 (T1655C, Leu552Pro). We believe that this alteration will affect the activity of the protein and this hypothesis is being tested by in vitro mutation analysis. Interestingly, only the precursor, not the mature form of the enzyme can be detected by Western blot analysis in this case, confirming our suspicion that a splicing mutation and a missense mutation are the probable cause of enzyme deficiency. In addition, we have not detected either of these changes in a population of 100 normal alleles ruling out the possibility of polymorphism. Our results further confirm the extensive genetic heterogeneity in the Caucasian patients affected with the infantile Pompe disease.



**Enhancing the sensitivity of mutation detection in tuberous sclerosis TSC1 and TSC2 genes by DHPLC, Primer Extension and Quantitative PCR analysis.** A. Allavena<sup>2</sup>, S. Padovan<sup>1,2</sup>, L. Longa<sup>2</sup>, A. Brusco<sup>2</sup>, C. Michielotto<sup>2</sup>, C. Cagnoli<sup>2</sup>, B. Ferrando<sup>2</sup>, M. Barberis<sup>2</sup>, N. Migone<sup>1,2</sup>. 1) CNR-CIOS, Torino; 2) Dept. of Genetics, Biology and Biochemistry, Torino University, Italy.

In a diagnostic setting, it is commonly observed that 20-30% of TSC patients does not reveal any mutation in either TSC gene following a single-step mutation screening. Low-level mosaicism, gene deletion, and cryptic mutation causing aberrant splicing or mono-allelic expression could partly account for these findings. In order to assess the relative contribution of the latter phenomena, we tested by DHPLC 199 TSC probands (75% sporadic, 25% familial): 156 probands (78%) showed pathogenic TSC mutations and 43 were mut-negative. Our multi-step strategy on DHPLC-neg cases is based on: a) Southern blot analysis, b) comparison of DHPLC and primer extension (PE) profiles at polymorphic sites with "reference heterozygotes" showing a 1:1 allelic ratio, c) quantitative fluorescent PCR to reveal mosaic or non-mosaic full gene deletions, wherever DHPLC and PE are inapplicable due to lack of heterozygosity and, d) PE adjacent to heterozygous sites on cDNA vs. DNA templates to detect unbalanced allelic expression. Our preliminary results are summarized as follows: Southern blot analysis allowed us to identify 20 partial/full gene deletions virtually undetectable by DHPLC. Two large TSC2 gene deletions were revealed by quantitative PCR only. PE on cDNA vs. DNA templates appeared precious in detecting two cases of unbalanced allelic expression. Overall, using this strategy, large rearrangements or cryptic mutations could be assigned in 24 of 43 apparently DHPLC-neg probands, enhancing the sensitivity of mutation detection (90%). So far only 1/2 of our mut-neg patients have been fully investigated by PE, PTT or quantitative PCR; it is likely that additional mutations will be identified in this subset, suggesting that the involvement of novel TSC loci should be confined to a minority of TSC probands. Work supported by Telethon, Italy (project E.730 ) and by "Assoc. E. & E. Rulfo per la Genetica Medica". M. Barberis is recipient of a fellowship from "Assoc. Italiana Sclerosi Tuberosa".

**Point mutations in the dystrophin gene: a super hot spot of mutation at a CpG dinucleotide and a difference in the pattern of microdeletions.** *C.H. Buzin<sup>1</sup>, J. Yan<sup>1</sup>, J. Feng<sup>1</sup>, J.R. Mendell<sup>2</sup>, S.S. Sommer<sup>1</sup>*. 1) Molecular Genetics, City of Hope Medical Center, Duarte, CA; 2) Ohio State University Medical Center, Columbus, OH.

About 35% of patients with Duchenne muscular dystrophy have point mutations in the dystrophin gene, one of the largest known genes that spans nearly 2.4 Mb of genomic DNA, contains 79 exons, and encodes a 14 kb transcript. Comprehensive mutation scanning was performed with DOVAM-S, a partially automated method that detects virtually all mutations. In this study, genomic DNA from 141 patients with DMD, ascertained both clinically and by muscle biopsy, was scanned in all coding exons, associated intronic splice regions, and promoter sequences. Although prior screening had excluded most patients with large deletion and duplication mutations, eight of the patients were found to have a large deletion. Of the remaining 133 patients, 98 (74%) had truncating point mutations, including nonsense, small deletions or insertions, and splice mutations. Of the 98 causative point mutations, 94 were independent as determined by haplotype analysis. No causative missense mutations were found, although many missense polymorphisms were discovered, all of which were seen also as second site changes in patients in which a truncating mutation was found. For frameshift and nonsense mutations (86 independent mutations), a mutation target could be determined within the coding region. One super hot spot mutation, a transition at CpG in exon 59 (arg2905ter), was detected in six patients, all with different haplotypes. When the point mutation types are normalized for their target, the relative rates of microdeletions/insertions, transitions and transversions at and not at CpG were similar to the factor IX gene. However, the size distribution of microdeletions was significantly different in DMD and factor IX.

**A Missense Mutation at Codon 846 in CRB-1 Gene Causes RP12 in a Pakistani Family.** *M. Ismail, A. Abid, A. Hameed, K. Anwar, S.Q. Mehdi, S. Khaliq.* Dr. A. Q. Khan Research Laboratories, Biomedical & Genetic Engineering Division, 24 Mauve Area, G-9/1, Islamabad, Pakistan.

Retinitis pigmentosa is clinically and genetically a group of inherited ocular diseases that involve progressive degeneration of photoreceptors resulting in the release of pigment by degenerating cells in the retinal pigment epithelium (RPE). It manifests initially as night blindness, followed by progressive loss of vision. RP12 is a severe and rare form of autosomal recessive RP characterised by a typical preservation of the para-arteriolar retinal pigment epithelium (PPRPE). Dysfunction of CRB-1 (human homolog of *Drosophila* cell-polarity gene 'crumbs') has been characterized as the cause for RP12. Several mutations have been reported in CRB-1 in a panel of unrelated RP12 patients including a homozygous AluY insertion. It has been suggested that it is an extracellular protein and probably plays a central role in localising the phototransduction complex to the apical membrane. A large Pakistani family was ascertained, showing typical autosomal recessive PPRPE phenotype. Linkage analysis of this family mapped the disease locus on chromosome 1q31-q32.1. Screening of the CRB-1 gene showed a homozygous G-A transition in exon 7 at nucleotide 2536 in all patients. Since the mode of inheritance was autosomal recessive, none of the parents (carrier) was homozygous for the mutation. The G-A transition results in the substitution of arginine for glycine at codon 846. This change has not been reported previously and could be the probable cause of RP in this family.

**The role of hPMS1 and hPMS2 in predisposing to colorectal cancer.** *T. LIU<sup>1</sup>, S. Kuismanen<sup>2</sup>, A. Percesepe<sup>3</sup>, ML. Bisgaard<sup>4</sup>, M. Pedroni<sup>3</sup>, P. Benatti<sup>3</sup>, M. Ponz de Leon<sup>3</sup>, P. Peltomki<sup>5</sup>, A. Lindblom<sup>1</sup>.* 1) Department of Molecular Medicine, Karolinska Institute, S171 76 Stockholm, Sweden; 2) Department of Medical Genetics, Haartman Institute, University of Helsinki, 00290 Haartmaninkatu, Helsinki; 3) Deptment of Internal Medicine, University of Modena, Viua del Pozzo, 71, 41100 Modena, Italy; 4) .Danish polyposis Register, Hvidover University Hospital, DK2100 Copenhagen, Denmark; 5) Human Cancer Genetics, Comprehensive Cancer Center, Ohio State University, 43210 Columbus Ohio, U.S.A.

Hereditary nonpolyposis colorectal cancer (HNPCC) is due to deficiency of mismatch repair. Inactivation of DNA mismatch repair underlies the genesis of microsatellite instability (MSI) in colorectal cancer. Germline mutations in three DNA mismatch repair genes, hMSH2, hMLH1, and hMSH6, have been found to segregate in HNPCC and HNPCClike families. The two DNA mismatch repair genes hPMS1 and hPMS2 have also been suggested to predispose to HNPCC. In this study 84 HNPCC and HNPCClike kindreds without a mutation in the other three known DNA mismatch repair genes, were screened for germline mutations in the hPMS1 or hPMS2 gene. No clear-cut pathogenic mutation was identified. Since the hPMS1 and hPMS2 genes were first reported in two HNPCC patients, germline mutations in hPMS2 have been demonstrated primarily in patients with Turcots syndrome. However, no mutation in any of the two genes has so far been found to segregate in HNPCC families. Until there is better evidence for an increased colorectal cancer risk associated to germline mutations in these genes, a conservative interpretation of the role of mutations in these genes is advised.

**FISH analysis and mutation screening of the Enhancer Of Zeste homolog 2 (EZH2) gene : a candidate gene for Coffin-Siris syndrome.** *A. Lossi<sup>1</sup>, M. Rahmoun<sup>1</sup>, D. Depetris<sup>1</sup>, M.G. Mattéi<sup>1</sup>, J. Bodurtha<sup>2</sup>, E. Lammer<sup>3</sup>, L. Villard<sup>1</sup>.* 1) Inserm U491. Faculté de Médecine La Timone. 27, Bd. Jean Moulin. 13385 Marseille Cedex 5. France; 2) Virginia Commonwealth University, Richmond VA 23298; 3) Genetics Center, Oakland Childrens Hospital, Oakland CA 94709.

Coffin-Siris syndrome (MIM #135900) is characterized by mental retardation, nail and phalanx abnormalities, and distinctive facial features. Most cases are sporadic, although a few familial cases have been described. The transmission mode is still debated. Two chromosomal rearrangements were described in patients with Coffin-Siris syndrome, both involving the 7q32-q34 region. These findings are compatible with a gene responsible for CSS located on chromosome 7. A specific interaction was reported between the XNP/ATR-X gene, responsible for ATR-X syndrome (MIM #301040) and the protein encoded by the enhancer of zeste homolog 2 gene (EZH2). EZH2 is located in 7q34. Given the presence of mental retardation and somewhat similar facial dysmorphism in both CSS and ATR-X patients, we questioned whether EZH2 could be involved in the etiology of CSS. In a first step, we used a genomic clone containing the EZH2 gene (clone RP5-1151\_m\_5) in FISH experiments to detect a potential chromosomal anomaly at this locus. Seven patients with a classical form of CSS were selected for this analysis. No deletion or other rearrangement were detected using the patients lymphoblasts for FISH experiments. We next screened the EZH2 gene for mutations in the seven patients. No disease-causing mutations were identified although a common polymorphism was detected. Taken together, these data probably rule out EZH2 as being the gene involved in Coffin-Siris syndrome.

**Hemochromatosis: Use of pyrosequencing for rapid detection of the most frequent mutations.** *P. Hilbert, A. Tricot, L. Van Maldergem, Y. Gillerot.* Inst.de Pathologie et de Genetique, Loverval, Belgium.

Hemochromatosis is a common autosomal recessive disorder of the iron metabolism. Different mutations of the HFE gene and recently of the TFR2 gene have been described associated with the disease. More than 80 % of the affected patients are homozygotes for the 845G --> A (C282Y) mutation of the HFE gene. However, depending on the population, 10-20 % of hereditary hemochromatosis may be due either to other HFE genotypes, particularly the compound heterozygous state for C282Y and the 187 C --> G (H63D) mutation, or to mutations of new other genes. Our aim was to develop a rapid and non expensive assay allowing us to rapidly screen our patients for the new mutations described in this disease. This method needed to be suitable to screen new patients as well as our collection of clinically affected patients previously tested negative for the C282Y and H63D mutations. We chose pyrosequencing to developed assays for the detection of mutations H63D, S65C, E168X, W169X and C282Y of the HFE gene as well as mutation Y250X of TFR2 gene. This new method allows rapid detection of SNPs . It is a sequencing-by-synthesis method in which a cascade of enzymatic reactions yields detectable light, which is proportional to incorporated nucleotides. One feature of typing SNPs with pyrosequencing is that each allelic variant will give a unique sequence compared to the two other variants. These variants can easily be distinguished by a pattern recognition software. The software displays the allelic alternatives and allows for direct comparison with the pyrosequencing raw data.

**Identification of a de novo mutation (P35S) for symphalangism in NOG gene.** *M. Mangino*<sup>1</sup>, *E. Flex*<sup>1</sup>, *M.C. Digilio*<sup>2</sup>, *A. Giannotti*<sup>2</sup>, *B. Dallapiccola*<sup>1,3</sup>. 1) C.S.S. Mendel, Rome, Italy; 2) Medical Genetics and Cardiology Bambino Gesù Hospital, Rome, Italy; 3) Dept. of Experimental Medicine and Pathology, University "La Sapienza" Rome, Italy.

Symphalangism (SYM1 [OMIM 185800]) is an autosomal dominant disorder characterized by early onset and progressive ankylosis of the proximal interphalangeal joints and conductive hearing loss. In 1999 Gong identified 5 dominant noggin gene mutations in unrelated families segregating proximal symphalangism and a de novo mutation in a patient with unaffected parents. The same authors also described a dominant NOG mutation in a family with multiple synostoses syndrome (SYNS1 [OMIM 186500]), demonstrating that SYM1 and SYNS1 are allelic disorders. We analysed an Italian family in which father and son had bilateral symphalangism of fingers 2-5 and toes 3-4, short first metacarpals, hypoplasia of distal phalanges of hands, and mild conductive hearing loss. The father had also thoracolumbar scoliosis. Facial appearance was unremarkable in both patients. We identified in our patient a de novo mutation (P35S) in the noggin gene. A different mutation in the same codon has been previously described by Gong et al. (P35R). Comparison between different noggin gene orthologs shows that codon 35 is conserved. Therefore this amino acid residue may play an important role in noggin gene function and is a mutation hot spot.

**Mutational analysis and phenotype-genotype correlation of the NOD2 gene in Crohn disease.** *S. Lesage<sup>1</sup>, H. Zouali<sup>1</sup>, J.P. Czard<sup>2</sup>, J. Belaiche<sup>3</sup>, S. Almer<sup>4</sup>, C. Tysk<sup>5</sup>, C.A. O'Morain<sup>6</sup>, M. Gassul<sup>7</sup>, R. Modigliani<sup>8</sup>, J.F. Colombel<sup>9</sup>, F. Merlin<sup>1</sup>, M. Chamaillard<sup>1</sup>, G. Thomas<sup>1</sup>, J.P. Hugot<sup>1</sup>.* 1) Fondation Jean Dausset, Paris, France; 2) Hopital Robert Debr, Paris, France; 3) Lige, Belgium; 4) Linkping, Sweden; 5) rebro, Sweden; 6) Dublin, Ireland; 7) Barcelona, Spain; 8) Hopital Saint Louis, Paris, France; 9) Hopital Calmette, Lille, France.

**Background** : Crohn's disease (CD) (MIM 266600) and ulcerative colitis (UC) (MIM 191390) are multifactorial diseases. Recently, the NOD2 gene was reported to be involved in CD susceptibility (Hugot et al, 2001; Ogura et al, 2001). **Aim** : to provide a detailed mutation analysis of the NOD2 gene in Inflammatory Bowel Disease (IBD) and genotype-phenotype correlation studies. **Patients and Methods** : Unrelated participants included 467 CD, 159 UC and 103 healthy controls. A complete questionnaire with detailed phenotype was obtained for all patients. The coding sequence of the NOD2 gene was entirely analysed by direct sequencing or using a Denaturated High Performance Liquid Chromatography (dHPLC) screening. ANOVA analyses and Chi square tests were used. **Results** : In addition to the 3 main variants (R765W, G881R, 980fs981X) known to be associated with CD, 65 additional variants were observed. These variants were significantly more frequent in CD than in UC and controls ( $p < 0,01$ ). They were found unfrequent in the exons encoding for the CARD domains. Considering only the potentially functional variations(s), 30% and 18% of CD patients had a single and double dose mutation, respectively. Genotype-phenotype correlation analyses argue for a stronger association of NOD2 to diseases with early onset (17 vs 20 years,  $p < 0,05$ ), ileo-caecal involvement ( $P < 0,001$ ) and complication by stenosis ( $P < 0,001$ ). **Conclusion** : Beside the 3 main variants of NOD2, several additional mutations are expected to play a role in CD predisposition. NOD2 mutations are more frequent in case of regional enteritis as described by Crohn in 1932.



**Complete-type X-linked Congenital Night Blindness (CSNB1): Novel NYX (Nyctalopin) mutations cause selective dysfunction of the ON-bipolar cell.** *K.T. Hirianna<sup>1</sup>, N. Khan<sup>1</sup>, E.L. Bingham<sup>1</sup>, P.A. Sieving<sup>1,2</sup>.* 1) Ophthalmology and Visual Sciences, Kellogg Eye Center, University of Michigan, Ann Arbor, MI; 2) National Eye Institute, NIH, Bethesda, MD.

CSNB1 is a recessive hereditary retinal disorder characterized by impaired visual sensitivity in very dim lighting. Associated ocular symptoms include myopia and nystagmus. The electroretinogram (ERG) shows a unique pattern of normal a-wave from rod and cone photoreceptors but a severely reduced b-wave from post synaptic retinal cells. Mutations in the NYX gene, that codes for nyctalopin and located at Xp11.23, have recently been identified in CSNB1 patients. We analyzed three "complete-type CSNB" families and identified two novel NYX gene mutations. One in exon 3 (803G→C) causes a missense change (R268P) affecting the Lucine Rich Repeat (LRR) 9 motif of the NYX protein. The second family has a two nucleotide change (710T→A;711C→A) that causes a missense change (L237Q) in LRR8 motif. The third Family has the same deletion of 24 nucleotides (85-108 del 24 nt.) in exon 3 that was previously reported for seven other CSNB1 families. We used sine-wave flicker ERG methods to attempt to localize the functional deficit in the neural pathways in the inner retina. We compared results in CSNB1 to ERG changes in monkey after applying glutamate analogs to the retina. We found that using DL-2-amino-4-phosphobutyric acid (APB) to block the sign-inverting synapse of cone photoreceptors on to the depolarizing (ON-) bipolar cells in monkey completely mimicked the human CSNB1 deficit, whereas using cis-2,3-piperidinedicarboxylic acid (PDA), an OFF-pathway blocker, gave quite dissimilar results. The APB treated monkey eyes show the unique loss of the normal flicker amplitude dip and the phase deflection near 12 Hz, as is also found in CSNB1 patients. These results for the first time localize the cone pathway dysfunction to the depolarizing bipolar cells of the ON-pathway of CSNB1 patients and exclude involvement of the hyperpolarizing (OFF-) bipolar cells. These results confirm the hypothesis developed earlier that retinal ON-pathway signaling is defective in complete-type CSNB.

**Refined Mapping of the Lanceolate Hair (*lah*) Gene.** A. Kljuic<sup>1</sup>, V.M. Aita<sup>1</sup>, A.A. Panteleyev<sup>1</sup>, J.P. Sundberg<sup>2</sup>, X. Montagutelli<sup>3</sup>, A.M. Christiano<sup>1</sup>. 1) Columbia University, Departments of Genetics & Development and Dermatology, New York, New York; 2) The Jackson Laboratory, Bar Harbor, Maine; 3) Institut Pasteur, Paris, France.

Lanceolate hair (*lah*) is an autosomal recessive mouse mutation characterized by generalized atrichia associated with the breakage of abnormal hair shafts. These hair shafts contain focal degeneration at their breakpoints with pronounced enlargement at the apex that resembles a lance head. The hairs are short and sparse and histological examination of anagen-stage follicles revealed abnormal cornification of the hair matrix. Initial genetic linkage and segregation analysis mapped the *lah* gene within a 5 cM region on mouse chromosome 18. We have further narrowed the interval by genotyping additional microsatellite markers within the region. The *lah* mutation arose in the PAS strain and has been backcrossed for 12 generations onto BALB/cByJ background. Thus, the markers were scored for retention of PAS specific alleles and critical recombination events were identified by the presence of BALB/cByJ alleles. The new critical interval is delineated by markers D18Mit21 and D18Mit223. Thus far, several genes involved in intracellular structures map to this region including laminin- $\alpha$ 3 and six desmosomal cadherin genes. We are currently screening potential candidate genes by Northern blot analysis and direct sequencing of cDNA templates.

**Molecular characterization of the LDL receptor gene in patients with familial hipercolesterolemia. M.T.**

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Familial Hypercholesterolemia (FH) is a common dominant autosomal disease which affects 1 in 500 individuals in most Western populations. It is characterized by high LDL (low density lipoprotein) plasma levels and is caused by a defect in the gene encoding the LDL receptor. In the human genome, the LDL receptor gene is located at the chromosome 19, being organized in 18 exons and it spans a region of about 60 kb. Gene analysis of the LDL receptor in patients with FH allowed the identification of more than 150 different mutations including insertions, deletions, and frameshift or nonsense mutations, distributed throughout the gene. Exon 4 (encodes the ligand-binding domain) keeps the majority of reported mutations. In this study, we have analyzed 66 patients with FH using two experimental methods (PCR amplification followed by automated sequencing in an ABI377™ sequencer). Thus far, we have investigated the first thirteen exons of the LDL receptor gene and we have detected four different point mutations, two of them synonymous and two non-synonymous. In the exon 4, there were identified the mutations G551A and C530T, that cause the aminoacid substitutions C163Y and S156L, respectively. The first mutation was identified in a patient and the second in another two unrelated patients. The two synonymous mutations identified were: T1773C (in the exon 12) and C1959T (in the exon 13). The parcial results presented here show that the experimental approach is appropriated for the molecular characterization of HF. Supported by: FAPESP & FUNDHERP.

**Design and development of a high-throughput test for Connexin 26 associated deafness using primer extension and mass spectrometric analysis.** *P. Merritt, H. Khoja, M.J. McGinniss.* Molecular Biology, Sequenom, Inc, San Diego, CA.

The incidence of congenital hearing loss is approximately 1 per 2,000 infants. Mutations in the GJB2 gene (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 the GJB2 gene (35delG) accounts for approximately two-thirds of all Cx26 mutations in DFNB1 patients. We have developed an assay using Sequenom's patented MassARRAY<sup>TM</sup> system that relies on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for automated genotyping and analysis. This assay is designed to genotype the two most common disease-associated mutations (35delG and 167delT) in a 5 ul biplex PCR and primer extension reaction optimized for 384-well format. We used this assay for genotyping 96 DNA samples, including 3 positive controls (35delG / 35delG, 35delG / 167delT and 35delG / wt) and 8 negative controls. 93 out of 96 samples were genotyped with 100% accuracy, and overall 96.8% assay success. Two samples were genotyped with low probability and one sample failed either due to failure of the extend reaction or it was not spotted on the mass spectrometric chip. No other unknown peaks were seen in this initial study. No carriers of the 35delG mutation were detected in this set of normal control samples. Direct measurement of primer extension products with mass spectrometry is a highly accurate, cost-effective and efficient method to detect common genetic forms of deafness such as Connexin 26. This MALDI-TOF MS method can now be further optimized and validated for clinical diagnostic testing in a high throughput setting.

**Molecular characterization of juvenile glaucoma.** *E. Heon*<sup>1, 2, 3</sup>, *A.L. Vincent*<sup>1, 2</sup>, *G. Billingsley*<sup>1, 2</sup>, *Y. Buys*<sup>1, 3</sup>, *G. Trope*<sup>1, 3</sup>, *M. Priston*<sup>1</sup>, *D. Williams-Lyn*<sup>1</sup>, *J. Sutherland*<sup>2</sup>, *A.V. Levin*<sup>2</sup>. 1) Ophthalmology, Vision Sci, Univ Toronto, Toronto, ON., Ontario, Canada; 2) Department of Ophthalmology, The Hospital for Sick Children, Toronto, On., Canada; 3) Department of Ophthalmology, The Toronto Western Hospital, Toronto, On., Canada.

Juvenile glaucoma (JOAG) refers to open angle glaucoma that is manifest between the age of 5 and 40 years of age. Glaucoma is genetically heterogeneous with at least three glaucoma genes identified. Although some cases of JOAG share a common genetic background with cases of adult-onset glaucoma, the natural history of JOAG is thought to be different. To better understand the basis of JOAG we did mutational analysis of three glaucoma related genes (MYOC, CYP1B1 and PITX2) in 65 cases affected with JOAG. The search for mutations was done using a combination of single strand conformation polymorphism (SSCP) and direct sequencing. Mutations were identified in 11% of JOAG cases. Mutations of MYOC were identified in 7 cases that included an individual with pigment dispersion syndrome and another family with cases of mixed glaucoma. Mutations in CYP1B1 were identified in three cases that did not have features suggestive of congenital glaucoma. No disease causing mutations was identified in PITX2. The phenotype-genotype correlations will be discussed. This work supports the important role of MYOC in JOAG but further demonstrates the variability in the expression of this gene. We report the association of MYOC mutations with mixed glaucoma and Pigment dispersion syndrome for the first time. This association of CYP1B1 with a non-congenital eye disorder, supports its role beyond congenital glaucoma and a potential role as a glaucoma-modifier gene.

**A PCR strategy for screening of  $\alpha$ -thalassemia mutations in Singapore.** *H.Y. Law<sup>1</sup>, G.P. Tan<sup>2</sup>, I. Ng<sup>1,2</sup>.* 1) Pediatrics, KK Women's and Children's Hosp, Singapore, Singapore; 2) National Thalassaemia Registry.

$\alpha$ -thalassemia is the most common genetic disease in Singapore, estimated to afflict 3-4% of the population which consists of Chinese (76.8%), Malay (13.9%), Indian (1.4%) and others (1.4%). A PCR strategy was established for the screening of various mutations that were identified in a systematic screen of  $\alpha$ -thalassaemia mutations in microcytic anemic patients attending the National Thalassaemia Registry, Pediatric outpatient and prenatal diagnosis clinics. The strategy consists of using 2 sets of multiplex PCRs for the detection of deletional mutations, and a Reverse Dot Blot (RDB) analysis for the detection of 5 point mutations: Hb Constant Spring(CS), Hb Pakae(PK) (Cd142T/C), Hb Quong Sze (QS) (Cd125CTG/CCG), Cd30(-GAG) and Cd59(GGC/GAC). All samples were first screened for 3 types of 2-gene deletion: South East Asian (SEA), Thai and Fil deletions. HbH samples and samples which gave negative results were also analysed by a multiplex PCR which detects 3.7kb and 4.2kb single gene deletions. Samples negative for both PCRs were then subjected to further RDB analysis for point mutations. Using this strategy, a total of 1292 unrelated individuals were screened and 1355 alleles analysed. While each ethnic group had it's characteristic frequency distribution of various mutations, the overall result showed that the SEA 2-gene deletion was the most common mutation(77.1%), followed by 3.7kb(14.9%) and 4.2kb(3.2%) single gene deletions, Thai 2-gene deletion(1.4%), HbCS(1.3%), HbQS(0.5%), Fil 2-gene deletion(0.3%) and HbPK, Cd30 and Cd59(0.1% each). Of the 11 alleles (0.8%) which were negative to all tests, 3 were found to have other point mutations by sequencing and 8 remained unidentified. In conclusion, 97% of the mutations were detected by 2 multiplex PCRs, and a futher 2% can be detected by RDB to achieve a 99% detection rate. This screening strategy is thus both efficient and effective in mutation identification which is important for the prevention and control of the disease.

**Congenital myasthenic syndrome due to a novel mutation in the acetylcholine receptor  $\epsilon$  subunit gene. V.**

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Congenital myasthenic syndromes (CMS) are genetically heterogeneous disorders caused by presynaptic, synaptic or postsynaptic defects. Previously described kinships with CMS showed a marked deficiency of acetylcholine receptors (AChR) at the neuromuscular junctions. We hereby, present our genetic findings in a 33-year-old woman from Greece with a classic CMS phenotype. Mutation screening revealed a novel 20 base pair homozygous deletion at position 4084 of the acetylcholine receptor epsilon subunit intron 11. Deleterious epsilon AChR mutations usually result in AChR deficiency. This novel mutation is currently under further investigation.

**Identification of a 40 kb deletion in 3q23 causing the Blepharophimosis, Ptosis and Epicanthus inversus**

**Syndrome (BPES).** *K. Lagerstedt*<sup>1</sup>, *G. Annerén*<sup>1</sup>, *S. Jagell*<sup>2</sup>, *L. Crisponi*<sup>3</sup>, *G. Pilia*<sup>3</sup>, *M-L. Bondeson*<sup>1</sup>. 1) Dept of Genetics and Pathology, Rudbeck Laboratory, Uppsala, Sweden; 2) Dept of Pediatrics, Gavle County Hosptial, Gavle, Sweden; 3) Istituto Regionale sulle Talassemie ed Anemie Mediterranee CNR, Dipartimento di Scienze Biomediche e Biotecnologie, Universita' degli studi di Cagliari, Cagliari, Italy.

The blepharophimosis, ptosis, epicanthus and inversus syndrome, BPES, is an autosomal dominant disorder affecting the craniofacial development. In type I, BPES is associated with female infertility while type II presents with the eyelid defects only. Recently, it was demonstrated that both types are associated with mutations in the FOXL2 gene located at 3q23. The FOXL2 gene encodes a putative winged helix/forkhead transcription factor where truncated proteins result in BPES type I and larger proteins in type II. Here, we report on the identification of a de novo deletion at 3q23 in a patient affected by BPES, mental retardation, microcephaly, fair skin and low set ears suggestive of a contiguous gene syndrome. We have analyzed the interval defining the deletion by using highly polymorphic markers and the fluorescent in situ hybridisation (FISH) technique. FISH analysis with twelve cosmids or PACs from around the FOXL2 gene showed haploinsufficiency for sequences corresponding to one of the cosmids analysed. From fiber-FISH analysis the size of the deletion was estimated to 40 kb. Cosmids and PACs located within the BPES critical region were mapped relative to the FOXL2 and the novel C3orf5 gene by STS content mapping. The deleted region was assigned to the C3orf5 gene that is located approximately 180 kb telomeric of the FOXL2 gene. No mutations were identified in the FOXL2 gene. The 40 kb deletion represents the smallest interstitial deletion reported so far associated with BPES, microcephaly and mental retardation. Our data suggest that the intragenic deletion in C3orf5 associated with BPES, microcephaly, mental retardation, ear dysmorphism and fair skin may exert position effect on expression of one or several genes in the BPES region.



**Characterization of two splice mutations provides conclusive evidence of the identification of the gene responsible for Bardet-Biedl syndrome type 4.** *K. Mykytyn<sup>1</sup>, A. Wright<sup>2</sup>, R. Riise<sup>3</sup>, E. Stone<sup>4</sup>, V. Sheffield<sup>1</sup>*. 1) Dept of Pediatrics, Howard Hughes Medical Institute, University of Iowa, Iowa City, IA; 2) MRC Human Genetics Unit, Western General Hospital, Edinburgh, Scotland; 3) Dept of Ophthalmology, Central Hospital, Hedmark, Hamar, Norway; 4) Dept of Ophthalmology, University of Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a heterogeneous autosomal recessive disorder characterized by obesity, pigmentary retinopathy, polydactyly, renal malformations, mental retardation, and hypogenitalism. The disorder is also associated with diabetes mellitus, hypertension and congenital heart disease. There are six distinct BBS loci. Although BBS is rare in most populations, there is considerable interest in identifying the genes causing BBS because components of the phenotype are common. Recently, we reported the positional cloning and identification of mutations in BBS patients in a novel gene designated *BBS4*. These homozygous mutations consisted of a missense mutation, an in-frame deletion predicting the loss of 48 amino acids, and two different splice site mutations. One splice mutation was a G>C transversion at the +1 position of the splice donor site in exon 4. The other was an A>C transversion at the -2 position of the splice acceptor site of exon 7. Although these mutations would be expected to result in abnormal splicing, we decided to confirm this by exploring the effect of the mutations on RNA processing. RNA from patient cell lines was isolated and used to synthesize cDNA for PCR analysis of the *BBS4* transcript. Sequencing of these PCR products demonstrated that the splice donor site mutation causes the skipping of exon 4 or exons 4 and 5, both of which are frameshift deletions. Sequencing of the transcript from the patient with the splice acceptor site mutation demonstrated aberrant splicing at a site five base pairs upstream of the original site, thereby introducing an insertion of five base pairs. The identification of the gene responsible for BBS4 will allow for the exploration of its function and the examination of potential interactions of the BBS4 protein with other BBS proteins and additional targets.

**Reevaluation of a genetic model for the development of exostosis in Hereditary Multiple Exostosis.** *J.T. Hecht<sup>1,3</sup>, C.R. Hall<sup>1</sup>, W.G. Cole<sup>2</sup>, R. Haynes<sup>3</sup>*. 1) Dept Pediatrics, Univ Texas Medical Sch, Houston, TX; 2) Department of Surgery, Hospital for Sick Children, Toronto, Canada; 3) Shriners Hospital-Houston, Houston, TX.

Mutations in the *EXT1* and *EXT2* genes cause Hereditary Multiple Exostosis (HME), a syndrome characterized by bony exostoses that develop juxtaposed to the growth plate. We and others have previously suggested that a two hit mutational model applies to the development of exostosis where a germline mutation coupled with a somatic mutation results in the loss of *EXT1* or *EXT2* function and subsequent aberrant chondrocyte proliferation and exostosis formation. We report the direct sequencing and LOH analyses of DNA from twelve HME exostoses from ten HME families, four solitary exostoses from individuals without HME, one Langer Gideon and one Trevor syndrome patient, and corresponding constitutional DNAs. All HME germline mutations were identified in both the exostosis and constitutional DNA samples. These mutations (85%) include two point mutations and three small deletions in the *EXT1* gene and one point mutation, one small deletion, and one small insertion in the *EXT2* gene. In addition, the two remaining (15%) HME exostosis samples were found by LOH and parental studies to have germline deletions of the *EXT1* or *EXT2* gene. Of the four solitary exostoses, only two were found to harbor mutations in the *EXT1* gene, which were not present in the constitutional DNA. Only a chromosome 8 deletion was identified in the constitutional and exostosis DNAs of the Langer Gideon patient, while no mutations were found in the Trevor patient. Of the eighteen exostoses screened, we find only one solitary case in which two somatic mutations, a deletion and LOH of the *EXT1* gene, is present. This provides limited support for the two hit hypothesis involving the *EXT1* and *EXT2* genes as a common mechanism for the development of exostosis. Alternative models based on the functional significance of the EXT proteins in heparan sulfate biosynthesis are suggested.

**A novel molecular diagnostic test for SHOX deficiency using mutation detection and SNP-based detection of whole gene deletions by DHPLC reveals new mutations.** *P. Marttila<sup>1</sup>, J. Ross<sup>2</sup>, A. Zinn<sup>3</sup>, S. Blakely<sup>1</sup>, J. Sernberger<sup>1</sup>, M. Stene<sup>1</sup>.* 1) Esoterix Endocrinology, Calabasas Hills, CA; 2) Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA; 3) McDermott Ctr Human Growth/Dev, University of Texas SW Medical School, Dallas, TX.

The SHOX (Short Stature Homeobox) gene defect causes Leri-Weill Dyschondrosteosis (LWD), impaired growth in some non-growth hormone deficient children, and short stature and skeletal features of Turner syndrome. LWD is a pseudoautosomal dominant disorder characterized by mesomelic dysplasia, short stature, and Madelung deformity. Most SHOX defects (70%) are whole gene deletions, the rest being point mutations.

We developed a highly sensitive and specific molecular diagnostic test for SHOX deficiency, named SHOX-DNA-Dx™. It detects both point mutations and whole gene deletions using heteroduplex analysis by DHPLC. Whole gene deletions are identified utilizing intragenic SNP's within the SHOX gene. SNP's were discovered by designing PCR amplicons throughout the SHOX gene and testing them for SNP content using DHPLC. The most informative SNP's were selected by screening 52 normal controls. Eight PCR amplicons were selected for SHOX-DNA-Dx. They include 2-5 SNP's or other polymorphisms per PCR amplicon. Heterozygosity in each PCR amplicon is high, varying from 33-88%. SHOX-DNA-Dx™ was validated by analyzing normal control samples, as well as subjects with short stature who were previously screened using FISH, and comparing the results.

We studied 12 unrelated LWD probands, and found whole gene deletions in seven, point mutations in four, and a partial deletion in one of the probands. This is the first time that partial deletion of SHOX has been reported. Three point mutations are new, previously unknown, and they all cosegregate with the LWD phenotype. In conclusion, SHOX-DNA-Dx™ is a highly sensitive and specific test for SHOX deficiency due to whole gene deletions, known or previously unknown point mutations in the SHOX gene.

**New Polymorphic Short Tandem Repeat for Diagnosis of the CMT1A Duplication and HNPP Deletion.** *K.S. Lee<sup>1</sup>, E.S. Park<sup>2</sup>, I.S. Seo<sup>1</sup>, B. Oh<sup>1</sup>, S.C. Jung<sup>1</sup>, J.S. Lee<sup>1,2,3</sup>.* 1) Biomedical Science, Nat'l Institute of Health, Seoul, Korea; 2) Brain Korea 21 for Biomedical Science, Yonsei University, Seoul, Korea; 3) Department of Pediatrics, Yonsei University College of Medicine, Seoul, 120-752, Korea.

Alterations in gene copy number may lead to under or over expression of these genes, resulting in the observed disease phenotype. Two well-documented diseases known to be caused by gene copy number alterations are the Charcot-Marie-Tooth type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP). CMT1A and HNPP are associated with dominantly inherited 1.5 Mb duplication or deletion at chromosome 17p11.2-p12, encompassing the peripheral myelin protein (PMP22) gene. Although several methods including southern blot, pulse-field gel electrophoresis, fluorescence in situ hybridization, real-time fluorescent PCR are useful to detect the DNA duplication or deletion, but they require labor-intensive or costly machines. Microsatellite marker typing is widely used for molecular diagnosis because of its convenience. In this study, we have developed a new microsatellite marker to improve the diagnosis of CMT1A/HNPP in Korean. The polymorphic short tandem repeat marker, (CA)<sub>n</sub> repeats, is located at about 3 Kb upstream region of PMP22 gene and shows more effective results than other markers in Korean CMT1A/HNPP patients. This microsatellite marker combined with other markers can be used for clinical application and for molecular diagnosis preferentially.

**Mutations of mitochondrial tRNA<sup>Leu</sup> and tRNA<sup>Lys</sup> gene in Korean patients with mitochondrial myopathy<sup>LeuLys</sup>.** *J-I. Kim<sup>1</sup>, K-S. Lee<sup>2</sup>, S-J. Jin<sup>2</sup>, S-K. Koo<sup>2</sup>, B. Oh<sup>2</sup>, S-C. Jung<sup>2</sup>, H-K. Lee<sup>3</sup>, J-S. Lee<sup>1,2,4</sup>.* 1) Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea; 2) Division of Genetic Disease, National Institute of Health, Seoul, Korea; 3) Department of Internal Medicine, Seoul National University School of Medicine, Seoul, Korea; 4) Department of Pediatrics, Yonsei University College of Medicine, Seoul, Korea.

Mitochondrial myopathies are associated with mutations in the mitochondrial or nuclear genome affecting mitochondrial function and frequently shown in mitochondrial diseases, such as mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), lebers hereditary optic neuropathy (LHON). Using PCR-direct sequencing analysis, we have investigated mitochondrial tRNA<sup>Leu</sup> gene for MELAS, tRNA<sup>Lys</sup> gene for MERRF and nucleotide 3460, 11778, 14484, 15257 mutations for LHON, which are known as major mutation sites in each case. In 7 of 66 cases, we identified four mutations at nucleotide A3243G, T3271C in tRNA<sup>Leu</sup>, A8344G in tRNA<sup>Lys</sup>, and G3460A. Two novel single nucleotide polymorphisms (SNPs) were also detected at nucleotide C3206T, C8431T. The low detection rate of the mutation in tested patients suggests that other neurologic diseases should be distinguished from MELAS and MERRF clinically. In addition, DNA analysis could be the choice of diagnostic method prior to muscle biopsy for mitochondrial myopathies.

**Mutation load in the mouse is age and tissue specific but not obviously related to rates of cell proliferation.** *K.A. Hill, A. Halangoda, P.W. Heinmoeller, C. Chitaphan, J. Wang, S.S. Sommer.* Molecular Genetics, City of Hope Ntl Med Ctr, Duarte, CA.

It is of interest to examine mutation load (mutation frequency, pattern and spectrum) with tissue type and age and identify mutational markers for aging and disease. The Big Blue® assay was used to measure mutation load in whole fetus (13.5 dpc) and eight tissues in post-natal mice at 10 days and 3, 10, 14, 17, 22, 25 and 30 months of age. There are three periods of mutagenesis: increasing mutagenesis during development, a plateau phase from early to mid-adulthood and a tissue specific increase in old age. Tissues that divide rapidly, moderately or not at all have quantitatively similar time courses of mutation from 10 d to 30 mo. Mutation load is unchanged from 3 to 30 mo in neurons and sperm, non-dividing and rapidly dividing tissues, respectively. The nondividing tissues, heart and adipose have increases in mutation load in old age, similar to the moderately dividing tissues, liver and kidney. Additional interesting findings include the following. 1) There is a single core mutation pattern in all tissues that is unaltered even when mutation frequencies change more than five fold. 2) Tandem GG to TT mutations occur in liver and adipose tissue and show increases with age and certain hotspots in liver. 3) Doublet mutations are enhanced over that expected for two independent events. 4) The distance between doublet mutations fits an exponential distribution consistent with their occurrence in the same cell cycle. The constancy of mutation load in sperm suggests that no advanced paternal age effect on mutation load will be found in mice; compatible with the absence of an advanced paternal age effect observed in the human factor IX gene (Hum Gen 105:629). It is significant to consider the difference in hypotheses related to mutational mechanisms for a three-step, tissue-specific profile of mutation load with age versus a constant linear increase in mutation load with age. Different life stages may have different mutagen exposures and different responses to antimutagens. These data provide a useful reference for future study of endogenous and exogenous mutagenesis.

**Krit1 mutations are responsible for the majority of inherited CCMs: a molecular genetic study of 38 families.** *D. Verlaan*<sup>1</sup>, *W.J. Davenport*<sup>1</sup>, *H. Stefan*<sup>3</sup>, *A.M. Siegel*<sup>2</sup>, *G.A. Rouleau*<sup>1</sup>. 1) Center for Research in Neurosciences, Dept. of Neurology, McGill University, Montreal, Quebec, Canada; 2) Epilepsy Program, Dept. of Neurology, University Hospital Zurich, Switzerland; 3) Kopfklinikum, University of Erlangen-Nurnberg, Germany.

Cerebral cavernous malformation (CCM) is a cerebral vascular lesion that can cause hemorrhagic strokes or seizures (MIM 116860). CCMs may arise sporadically or be dominantly inherited. Currently, three loci have been mapped, CCM1 (7q21-22), CCM2 (7p13-15) and CCM3 (3q25.2-27), accounting for 40%, 20% and 40% respectively of families affected with the disorder. The Krit1 gene has been identified as the gene causing CCM1. Recently, the presence of four additional upstream coding exons of the Krit1 gene has been predicted. In order to confirm the existence of these exons and determine the relative frequency with which Krit1 mutations cause CCM, we screened the gene for mutation by SSCP in 38 families participating in the International Familial Cerebral Angioma Study (IFCAS). We have found two new mutations in these novel upstream exons confirming the role of these exons in the disorder as well as six additional mutations in the previously known exons. Results indicate that approximately 50% of our CCM families tested have a mutation in this gene, indicating that the percentage of CCM caused by Krit1 mutations may be higher than previously thought.

**A G>T Substitution 7 bp downstream from the *CPT2* gene polyadenylation site associated with carnitine palmitoyltransferase II deficiency.** *G.D. Vladutiu<sup>1</sup>, D.R. Smail<sup>1</sup>, L. Gambino<sup>1</sup>, M.J. Bennett<sup>2</sup>*. 1) Dept Pediatrics, SUNY Buffalo, Buffalo, NY; 2) Dept Pathology & Pediatrics, Univ of Texas Southwestern Medical Center, Dallas, TX.

The polyadenylation region of nearly all eukaryotic pre-mRNAs contains important primary (AAUAAA and GU-rich sequences) and secondary structures (stem-loop) necessary for the binding of polyadenylation factors such as CPSF and CstF. These proteins are important for proper 3' end cleavage and addition of the poly(A) tail. Mutations in genomic DNA encoding the polyadenylation region can adversely affect gene expression by altering the structure of the pre-mRNA, and its ability to be bound by such proteins. A patient with myopathic CPT II deficiency (8% of normal activity in fibroblasts) was screened for the 6 most common mutations in the *CPT2* gene and found to be heterozygous for the S113L mutation. DNA sequencing of all 5 exons, 650 bases of flanking intronic sequence, 890 bases of the promoter region and 130 bases beyond the polyadenylation site identified a novel base pair substitution (PAS+7 G>T) 7 nucleotides downstream of the polyadenylation site. PAS+7 G>T was not found in 50 individuals from the general population, nor in 43 patients with varying degrees of CPT II deficiency. A third polymorphism, PAS+11 T>C (general population: T=0.62; C=0.38) for which the study patient was TT, exists 4 bp downstream from the new mutation. In preliminary studies, the frequency of the T allele was increased (T=0.85) in individuals with significant CPT II deficiency. Both PAS+7 G>T and PAS+11 T>C occur within the GU-rich sequence, yet PAS+7 G>T appears to be more disruptive to the predicted stem-loop target of the CstF protein than the polymorphism. Based on our findings, it may be hypothesized that PAS+7 G>T is a pathogenic mutation that impairs polyadenylation by altering the secondary structure of pre-mRNA. This is the first report of a mutation outside the *CPT2* gene coding region that appears to be causative for CPT II deficiency demonstrating the need for thorough screening of the entire gene region in suspected cases.



**Keratin-9 gene mutation in a family with Epidermolytic Palmoplantar Keratoderma.** *L. Peleg<sup>1</sup>, B. Goldman<sup>2</sup>, M. Karpati<sup>1</sup>, B. Amichai<sup>1</sup>*. 1) The Danek Gertner Institute of Human Genetic, Sheba Medical Ctr, Tel Hashomer, Israel; 2) Department of Dermatology, Huzot Clinic, Kupat Holim, Ashkelon, Israel.

Voerners Epidermolytic palmoplantar keratoderma (EPPK) is one form of a heterogeneous group of dermatoses, most frequently inherited in an autosomal dominant manner. The gene associated with EPPK was mapped to 17q11-23, to the type 1 (acidic) keratin gene cluster. In 1993 the cDNA of Keratin 9 (KRT9) was cloned and sequenced and the resulting polypeptide was found to be expressed in the epidermis of palms and soles. Most of the mutations with a clear association to EPPK have been located within residues 156-171 of the KRT9 polypeptide. We studied four affected and two non-affected individuals of three generations of an Ashkenazi Jewish family from Russia, suffering from EPPK. In addition DNA samples of two non-related healthy controls were also analyzed. An A to G substitution at position 545 was identified by sequencing a 200 bp fragment (bp 497-697), generating an amino acid substitution of asparagine in position 160 to serine. Since this mutation does not alter the recognition site of any known restriction enzyme, its presence was confirmed by sequencing all the studied DNA samples. The mutation was restricted to the four affected individuals' DNA and was not found in the four healthy individuals. As this mutation was already reported in an Austrian-Alpine family with EPPK, an analysis of the polymorphic CA repeat region in intron 4 of the gene was undertaken. In the Austrian family the disease was associated with an allele containing 21 repeats. In the present family (six members) four alleles were revealed: 17, 20, 22 and 23 CA repeats. The mutation 545A to G was associated with the allele containing 20 repeats. These results suggested that different ancestors introduced the mutation in the 2 families.

**Identification of ABCC6 pseudogenes on human chromosome 16p: Implications for mutation detection in pseudoxanthoma elasticum.** *L. Pulkkinen, A. Nakano, F. Ringpfeil, J. Uitto.* Dept Dermatology and Cutaneous Biology, Thomas Jefferson Univ, Philadelphia, PA.

Pseudoxanthoma elasticum (PXE), a heritable disorder affecting the skin, eyes and the cardiovascular system, has been recently linked to mutations in the ABCC6 gene on chromosome 16p13.1. The original mutation detection strategy employed by us consisted of amplification of each exon of the ABCC6 gene with primer pairs placed on the flanking introns, followed by heteroduplex scanning and direct nucleotide sequencing. However, this approach suggested the presence of multiple copies of the 5'-region of the gene when total genomic DNA was used as template. In this study, we have identified two pseudogenes containing sequences highly homologous to the ABCC6 5'-end. First, by the use of allele specific PCR, two BAC-clones containing a putative pseudogene of ABCC6, designated as ABCC6-y1, were isolated from the human BAC-library. Sequence analysis of ABCC6-y1 revealed it to be a truncated copy of ABCC6 which contains upstream region as well as exon 1-intron 9 segment of the gene. Secondly, homology search of a high-throughput sequence (htgs) database revealed the presence of another truncated copy of ABCC6, designated as ABCC6-y2, which was shown to harbor upstream sequences as well as a segment spanning exon 1 through intron 4 of ABCC6. In addition to several nucleotide differences in the flanking introns and the upstream region, both pseudogenes contain several nucleotide changes in the exonic sequences, including stop codon mutations, which complicated mutation analysis in patients with PXE. Nucleotide differences in flanking introns between these two pseudogenes and ABCC6 allowed us to design allele specific primers which eliminate the amplification of both pseudogene sequences by PCR, providing reliable amplification of ABCC6-specific sequences only. The use of allele specific PCR has revealed thus far two pathogenetic 5'-end PXE mutations, 179del9 and T364R in exons 2 and 9, respectively, as well as several polymorphisms within the upstream region and exons 1-9 of ABCC6.

**Patients definitively diagnosed with TSC yield a higher mutation detection rate.** A.J. Tucker<sup>1</sup>, H. Northrup<sup>1</sup>, C.J. Ohl<sup>1</sup>, E.S. Roach<sup>2,3</sup>, S.P. Sparagana<sup>2,3</sup>, M.R. Delgado<sup>2,3</sup>, L.L. Batchelor<sup>2</sup>, K.S. Au<sup>1</sup>. 1) The University of Texas-Houston Medical School, Houston, TX; 2) Texas Scottish Rite Hospital for Children, Dallas, TX; 3) The University of Texas Southwestern Medical School, Dallas, TX.

Tuberous sclerosis complex (TSC) is an autosomal dominant human disease characterized by hamartomas and hamartias in multiple tissue organs. A mutation in either the TSC1 or TSC2 gene is causative for this condition. Diagnostic criteria have been developed to aid physicians in diagnosis, utilizing a classification of *Definite*, *Probable* or *Possible* diagnoses. Familiarity with these criteria may vary with physician expertise or background. Previous attempts to develop clinical DNA testing protocols have utilized mutation-screening methods with detection rates ranging from 58% (heteroduplex analysis) to 68% (DHPLC). This study employed a new protocol of direct sequencing of PCR products from all TSC1 and TSC2 coding exons. A group of 49 patients have been sequenced to date, including 10 that had screened negative by SSCA. The remaining 39 patients included 20 with documented *Definite* diagnoses referred by the TSC clinic at Texas Scottish Rite Hospital (TSRH) and 19 (15 *Definite* and 4 *Possible* or non-specific criteria) with undocumented diagnoses referred by various sources. Upon sequencing 52 of the 62 most frequently mutated coding exons, three likely disease-causing mutations were identified among the 10 patients previously studied, indicating a likely increased sensitivity of direct sequencing. In the 20 TSRH patients, 17 likely disease-causing mutations were identified, resulting in a detection rate of 85% in this group. In contrast, likely disease-causing mutations were found in 11 (9 *Definite*, 2 *Possible*) of the 19 remaining patients, with a detection rate of 57.9%, significantly lower than that for the TSRH group ( $p < 0.001$ ). The detection rate in patients with undocumented *Definite* diagnoses was 60%, again significantly lower than that of TSRH cases ( $p < 0.01$ ). These findings suggest the consistent application of clinical diagnostic criteria by those trained in TSC diagnosis will yield a more definitive diagnosis, therefore yielding a higher rate of mutation detection.

**Biplex Invader® assays for simple, accurate detection of factor V Leiden and other thrombosis risk factors.** *E.B. Rasmussen<sup>1</sup>, D.E. Burris<sup>1</sup>, E.K. Dorn<sup>1</sup>, S.S. Wigdal<sup>1</sup>, D. Arco<sup>1</sup>, P. Agarwal<sup>1</sup>, R. Selzer<sup>2</sup>, K. Hogan<sup>2</sup>, B. Neri<sup>1</sup>, R. Kwiatkowski<sup>1</sup>. 1) Third Wave Technologies, Inc., Madison, WI; 2) Department of Anesthesiology, University of Wisconsin - Madison, Madison, WI.*

The factor V Leiden mutation is one of several genetic variations linked to an increased risk of deep vein thrombosis (DVT). Some polymorphisms, such as those affecting the factor V and factor II genes, disrupt the normal functioning of the coagulation cascade by acting directly on key players within that biochemical pathway. Other polymorphisms act through ancillary pathways, such as mutations within the methylenetetrahydrofolate reductase (MTHFR) gene. Such mutations can lead to elevated levels of plasma homocysteine or hyperhomocysteinemia, a secondary risk factor for DVT. Accurate diagnosis of these and other risk factors is essential for the proper management of patients at a heightened risk for thrombotic or embolic complications.

Using our proprietary Invader technology, we have produced analyte specific reagents for use in the genetic detection of a number of thrombotic and cardiac risk factors, including polymorphisms in factor V [Leiden (G1691A)], factor II [G20210A], MTHFR [C677T and A1298C], Gp IIIa [PLA1/2 (T1565C)], and Apolipoprotein E [E2, E3, and E4 Isoforms], with still others under development. Assays using these reagents are simple, requiring only a single post set-up addition, and fast, producing a result directly from genomic DNA in just over four hours without the need for target amplification by PCR. Detection is straightforward; the reaction is isothermal and homogeneous, and the biplex format allows for the assessment of both wild-type and mutant alleles in a single reaction well. Assays are run in a standard 96-well microtiter format and the results are read directly by any fluorescence plate reader capable of detecting two spectrally-distinct fluorophors.

In this study, we demonstrate the application of Invader reagents to the biplex detection of these thrombotic and cardiac risk factors across a panel of human subjects.

***MECP2* gene analysis in Japanese patients with classical and variant Rett syndromes and with Rett-like features.**

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The Rett syndrome (RTT, MIM 312750), an X-linked dominant neurodevelopmental disorder, is caused by mutations in the methyl-CpG-binding protein 2 gene (*MECP2*). We have analyzed the gene in 37 Japanese patients divided into classical RTT (14 cases), variant RTT (13 cases), and mentally retarded patients with Rett-like features (10 cases). Mutations in *MECP2* were identified in most of the patients with classical and variant RTT (25 of 27 cases). Six reportedly common mutations including 3 missense mutations (R133C, T158M, and R306C) and 3 nonsense mutations (R255X, R270X, and R294X) were detected in 17 cases. Rare single nucleotide substitutions, Y141X, D156E and P225R, were also each identified in one patient, respectively. In addition, one insertion mutation (1189insA resulting in 397fs404X) and four deletion mutations, including one double deletion (451delG resulting in 151fs159X, 100del4 resulting in 34fs124X, 1124del53 resulting in 376fs386X, and 881del289 plus 1187del8 resulting in an insertion of 6 different amino acids instead of deleted 105 amino acids) were newly identified. In the 10 mentally retarded patients with Rett-like features, however, no mutations were detected in the coding region of *MECP2*. The finding of *MECP2* mutations in 92.5 % of patients with RTT indicates that RTT fulfilling the diagnostic criteria are due to genetic alteration. Although no significant genotype-phenotype correlations were apparent, some tendencies were observed regarding symptoms like microcephaly and an inability to walk. First, the patients without microcephaly were limited to those with missense mutations or a deletion at CTR. Second, in most of the patients who able to walk, the mutations were found downstream from TRD of *MECP2*, except in 3 cases with MBD missense mutations.

**A novel Q378X mutation exists in *ABCC6* and its pseudogene - Implications for mutation analysis in pseudoxanthoma elasticum (PXE).** B. Struk<sup>1, 7</sup>, A. Lumsden<sup>2</sup>, L. Cai<sup>1</sup>, U.P. Guenther<sup>7</sup>, S.A. Neldner<sup>1</sup>, S. Zäch<sup>3</sup>, R. Ramesar<sup>4</sup>, D. Hohl<sup>3</sup>, D.F. Callen<sup>2</sup>, K.H. Neldner<sup>5</sup>, R.I. Richards<sup>2</sup>, K. Lindpaintner<sup>1, 6, 7</sup>. 1) Endocrinology and Hypertension Division, Brigham and Women's Hospital, Boston, MA; 2) Department of Cytogenetics and Molecular Genetics, Women's and Children's Hospital, N. Adelaide SA, AUS; 3) Department of Dermatology, University of Lausanne, Lausanne, CH; 4) Department of Human Genetics, Medical School, University of Cape Town, SA; 5) Department of Dermatology, Texas Tech University Health Sciences Center, Lubbock, TX; 6) F. Hoffmann-La Roche Ltd, Roche Genetics, Pharmaceuticals Division, Basel, CH; 7) Max-Delbrueck-Centrum for Molecular Medicine, Berlin, FRG.

PXE is an inherited disorder of the elastic tissue with progressive calcification of elastic fibers in skin, eye, and the cardiovascular system. Recently, mutations in the *ABCC6* gene were identified as cause of PXE. We had previously identified the starting point of a large genomic segmental duplication within the PXE locus in the cytogenetic interval defined by the *Cy19* and *Cy185* somatic cell hybrid breakpoints on chromosome 16p13.1. We more precisely mapped this duplication to include exons 1 to 9 of *ABCC6*. Sequence and RFLP analysis for exon 9 in all members from the PXE families and in 192 controls revealed either a homozygous or heterozygous state for the Q378X (C1132T) nonsense mutation in all individuals, suggesting that one or several copies of an *ABCC6* pseudogene (*yABCC6*) lie within this duplication. At least one copy contains exon 1 to 9 and maps to *Cy163*, but not to *Cy11*. This copy and/or an additional copy of *yABCC6* within *Cy19-Cy183* carry the Q378X mutation that masks its correct identification as being causative in PXE. Long range PCR of exon 9 from sequence outside the genomic replication circumvents interference from the *yABCC6* DNA sequences and demonstrates that the Q378X mutation in the *ABCC6* gene is associated with PXE in some families.

These findings lead us to propose that gene conversion from *yABCC6* to *ABCC6* plays a functional role in mutations causing PXE.

**Relevance of exon 15 mutations in PKD1 disease.** *R.A. Perrichot<sup>1,2</sup>, A. Grall<sup>1</sup>, B. Mercier<sup>1</sup>, A. Carre<sup>1</sup>, I. Quere<sup>1</sup>, J. Cledes<sup>2</sup>, C. Ferec<sup>1</sup>.* 1) Genetic Laboratory, ETSBO-CHU, Brest, France; 2) Nephrology department, CHU la Cavale Blanche, Brest - France.

PKD1, the major gene involved in the transmission of autosomal dominant polycystic kidney disease is actually subjected to intensive molecular analysis all around the world considering the frequency of the disease and the number of patients requiring renal replacement therapy. The molecular analysis of PKD1 is especially not easy undertaking since two thirds of the gene is repeated a number of times on the proximal part of the short arm of chromosome 16, however the identification of locus-specific primers that can selectively amplify PKD1 but not its homologues has enabled the analysis of the 5' region of the gene. Both our group and others have contributed to the identification of more than 150 mutations in this gene, nevertheless the mutation detection rate remains disappointingly low in each group. Using a step of long-range amplification of exons 11-15 from genomic DNA of French ADPKD patients, we performed a DGGE screening for mutations in this PKD1 region. We identified about 20 DNA variants including 11 novel mutations and several polymorphisms. If we take into account these results, to our knowledge 167 mutations have been reported in PKD1 gene up to now; among them 44 (26.34%) are located in exon 15. This rate will be probably higher in a few years when all groups working in this field will achieve their mutation screening. Unfortunately one could not exclude a bias in this analysis, linked to the large size of this exon; however this region of the gene seems to be functionally important binding the PKD domains (previously named immuno-globulin like domains) supposed to play an important role in cell-cell and cell-matrix interactions as suggested recently in vitro. The loss of intercellular interactions due to a mutated protein can be an important step in cystogenesis process actually not yet understood. The occurring of mutation in this region were recently associated with more severe phenotype, however before considering the relationship between PKD1 genotype and phenotype we must extend our mutation screening to the whole coding sequence.

**Evaluation of PCR enzyme related parameters affecting Denaturing High Performance Liquid Chromatography.**

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Denaturing High Performance Liquid Chromatography (DHPLC) has gained significant popularity as a method for identifying mutations. Routinely, PCR from genomic DNA samples followed by heteroduplex formation in the presence of a mutation enables resolution of homoduplex and heteroduplex species using Ion Paired Reverse Phase HPLC and the DNASep® Cartridge technology (Transgenomic Inc, CA). A variety of PCR enzymes have been used in conjunction with the DHPLC application to successfully locate both unknown mutations and develop diagnostic genotyping applications. The same principle enabling the discovery of sequence changes in DNA using the DNASep® Cartridge technology and the WAVE® Nucleic Acid Fragment Analysis System allows analysis of the differing PCR enzyme related parameters affecting the quality of DHPLC results. Primarily, the application specific PCR enzyme misincorporation rate can be compared between enzymes since individual misincorporation events throughout the PCR amplification process will be present during subsequent DHPLC sample analysis. The misincorporation events appear as a random low-level heteroduplex peak, which can be comparatively quantified. Secondly, the high throughput nature for DHPLC related applications (SNP mapping and diagnostic genotyping for example) requires a high level of reproducibility, which can be enhanced by PCR component compatibility. One of the major contributors to sample to sample variability lies in the compatibility of PCR buffer constituents in relation to the DNASep® matrix. These two main factors affecting DHPLC related methodologies are examined here for a variety of routinely used PCR enzyme preparations.



**SNP identification, linkage disequilibrium (LD), and maternal origin of mutations within the TSC2 gene.** *P.S.*

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Inactivating mutations in the TSC2 gene, consisting of 41 coding exons in 40 kb on 16p13, cause the hamartoma syndrome tuberous sclerosis. We have identified over 250 mutations in the TSC2 gene (e.g. AJHG 2001, 68:64-80). During this analysis we also identified 10 SNPs that occur within exons or close to exon boundaries at minor allele frequencies of > 5%. The last six of these SNPs (minor allele freq. 9-27%) and the microsatellite marker kg8 occur within a region of 600 bp that contains exons 40 and 41; exons 38 and 39 are also nearby. The other four SNPs are found 23-33 kb from this cluster. We genotyped the 6 SNPs and kg8 in a set of 39 Caucasian and 1 African parent-child trios, and determined haplotypes by Mendelian segregation, with cloning as needed. 26 unique haplotypes were determined in the 160 chromosomes of the parents, with the most common haplotypes accounting for 53%, 11%, 6%, and 5% of chromosomes. 12 of 15 pairwise comparisons among the 6 SNPs showed strong LD with  $D' > 0.8$ ; the other 3 had  $D' 0.54-0.68$ . Exons 38-40 are relative hotspots for mutation in TSC2, and we have identified 30 families with small mutations in this region, and 9 families with large genomic deletions that include this region. The haplotypes on which these mutations occurred was determined by cloning and sequencing, and family studies. The distribution of 38 mutation-bearing haplotypes was similar to that of the trio parents, indicating that there was no haplotype that predisposed to mutation in this region of TSC2. When possible we performed family analysis in sporadic cases to determine the parent of origin of mutation. In 7 cases (5 sm mtns, 2 deletions) the mother was the parent of origin, while in 2 cases (both sm mtns) the father was the parent of origin. Thus, mutations in TSC2 are predominantly of maternal origin, in contrast to most other genetic disease.

**Haplotype analysis of tyrosinase gene in OCA1.** *J.E. Pietsch<sup>1</sup>, S. Shriram<sup>1</sup>, J.P. Fryer<sup>1</sup>, R.A. King<sup>1,2</sup>, W.S. Oetting<sup>1,2</sup>.*  
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**Background:** Mutations of the tyrosinase gene produce oculocutaneous albinism type 1 (OCA1). Most affected individuals are compound heterozygotes with different maternal and paternal mutations, and the majority of the identified mutations have been sequence changes in the coding region or adjacent introns. Some individuals with OCA1 have only one of the two mutations identified, however, suggesting another type mutation responsible for loss of function of this allele. We hypothesize that regulatory regions of this gene beyond those currently characterized may be responsible for this loss of function, and therefore have extended our analysis of the 5 promoter region of this gene.

**Methods:** 4000 bp of the 5 promoter region of the tyrosinase gene was sequenced in DNA from 11 individuals with OCA1 who had 1 of 2 mutations identified. Specific parts of this region were then sequenced in DNA from an additional 6 families containing a child with OCA1. **Results:** Four single nucleotide polymorphic (SNP) changes were identified: -3879 (g ® a), -3748 (a ® c), -3421 (g ® a), and 3046 (g ® a). A polymorphic LINE insertion was also identified at 2093. Extended haplotypes were constructed with the 4 new SNPs, the insertion, and the known polymorphisms at 301 (c®t), -199 (c ® a), codon 192 (C ® A), codon 402 (G ® A) and the Taq I site in intron 1. One extended haplotype was found in 6/9 individuals with OCA1 and only 1 of 2 identified mutations. In all cases, the haplotype represented the allele without the mutation. The haplotype was not found in the parent who carried the mutation nor in individuals with both mutations identified. **Conclusion:** We have identified a haplotype of the tyrosinase gene in OCA1 that has no pathologic sequence change in the coding region but is associated with the loss of pigmentation. The mutation(s) responsible for inactivation of this allele appears to be in disequilibrium with this haplotype but has not been identified.

**Usher Syndrome type I mutations in MYO7a and Harmonin.** *M.D. Weston, L.M. Astuto, C.A. Carney, W.J. Kimberling.* Dept Genetics, Boys Town National Res Hosp, Omaha, NE.

Usher syndrome type I (USH1) is a genetically heterogeneous autosomal recessive disease of congenital profound hearing loss, vestibular areflexia and retinitis pigmentosa. Six genetic subtypes (USH1A-F) and 3 genes have been discovered to cause the disease. The most prevalent genetic subtype, USH1B, is caused by mutations in MYO7A. The causative genes for USH1C, USH1D and USH1F have been shown to be the harminin, CDH23 and PCDH15. As these and other genes involved in Usher Syndrome are discovered, an accurate molecular diagnosis of the causative gene defect will be an important variable in evaluating the benefit of existing and new treatment options for slowing and/or arresting retinal deterioration. The accuracy of genetic counseling of recurrence risk estimation will also be increased as the distribution and frequencies of mutations is more accurately estimated ethnically and geographically. Towards this end, clinical cases of USH1 have been screened for mutations in 3 of the known genes. Based on tests of genetic heterogeneity using linkage data, 70% of Usher I cases were compatible with linkage to 11q with the majority of the rest showing 10q linkage, the location for both USH1D (CDH23) and USH1F (PCDH15). From an original cohort of 152 clinical cases of Usher type I, mutation screening in MYO7a diagnosed ~40% (67/152) of these cases. Results of CDH23 and Harmonin mutation screening to date have diagnosed an additional ~9% (13/152) and ~4% (6/152), respectively, from this same cohort. The previously published MYO7a missense mutations R1602Q and Y1719C, are questionable in producing the Usher phenotype, based on cis linkage to other more highly conserved missense mutations and/or their presence in control populations.

**Possible involvement of interleukin 10 receptor alpha (IL10RA) in Familial Enteropathy with Villous Edema.**

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Familial Enteropathy with Villous Edema and immunoglobulin G2 deficiency (FEVE; MIM 600351), is an autosomal dominant disorder that typically manifests in childhood as a recurrent acute, life-threatening secretory diarrhea associated with distinctive jejunal histologic changes and IgG2 subclass deficiency. Symptoms typically begin abruptly with anorexia and vomiting, and rapidly progress to massive secretory diarrhea and shock with profound neutropenia and hypoproteinemia, including hypoalbuminemia and hypogammaglobulinemia. We previously reported the results of a genome-wide screen for the FEVE locus, in which a 12.5cM region on chromosome 11q23 was identified with a LOD score of 6.2. Subsequent work using a higher density marker array has reduced the critical interval to about 9 cM. Several candidate genes map to this region and we have initiated sequencing in parallel with ongoing linkage analysis.

The CD3-delta subunit, which maps to the FEVE region, has been previously reported to be associated with intestinal malabsorption. Sequencing of this gene in affected FEVE family members revealed a C->G transversion, but this base change in intron 4 (IVS4+60) is not predicted to affect exon splicing.

Interleukin 10 down-regulates the inflammatory response, and protects against the lethality of intra-abdominal infection and sepsis. Interleukin 10 receptor alpha maps to the FEVE critical region. Sequencing of this gene revealed an A->G transition in exon 2 resulting in a Serine to Glycine missense substitution (S101G). This mutation segregates with the condition in this family, and has not been found in any unrelated controls. The mouse protein sequence contains a glutamine at this position, with extensive proximal sequence homology. This mutation is currently unclassified, but work is ongoing to determine whether IL10RA may play a direct role in FEVE.

**Genotype frequencies of C282Y and H63D alleles in the Hemochromatosis (HFE) gene from 16,822 patient samples submitted to a reference laboratory for HFE genotyping.** *W. Sun, A. Buller, N. Huynh, S. Huang, M. Vu, L. Avila, C. Chen, B. Bower, S. York, T. Entwistle, C. Strom.* Molec Gen Dept, Nichols Inst, Quest Diagnostics Inc, San Juan Capistrano, CA.

Hereditary hemochromatosis (HH) is one of the most common autosomal recessive genetic diseases in the US affecting between 1:200 - 1:500 people. Two missense mutations (C282Y and H63D) in the HFE gene have been associated with HH. This study investigated genotype frequencies of these mutations in samples sent to a reference laboratory for HFE genotyping. It is assumed these patients have symptoms of iron overload and / or positive family histories of HH. These data are important for quality assurance monitoring of HH testing and for clinicians to be aware of the percentage of such patients who will actually have HFE mutations.

Quest Diagnostics / Nichols Institute has been performing HH genotyping since 12/99. Since that time 16,839 specimens were submitted and 16,822 (>99.8%) were successfully genotyped. We compared our data to those of a recent study (Steinberg et al, JAMA 285:2216-2222) reporting the frequency of these alleles in an unselected US population cohort of 5,171 individuals. Our population had a 53 fold increase in the frequency of homozygous C282Y (14% vs 0.26%), with more modest elevations (3.6 fold and 1.8 fold) in the frequency of compound heterozygotes for C282Y / H63D (7.1% vs 1.97%) and homozygotes for H63D (3.5% vs 1.89%).

Several studies have demonstrated the prevalence of C282Y homozygotes in patients with HH. These data provide further evidence that the C282Y mutation is an important contributor to HH. The minimal enrichment of patients with H63D mutations in the patient population sheds further questions on whether this allele is a significant contributor to iron overload syndromes despite the published observation of a significantly increased risk of HH in homozygotes for H63D.

**Screening a human male infertility candidate gene, *BOULE*, to identify sequence variants in infertile men.** *E.Y. Xu<sup>1</sup>, D. Lee<sup>1</sup>, P. Turek<sup>2</sup>, R.A. Reijo Pera<sup>1</sup>.* 1) OBGYN and Reproductive Science, Univ. of California-San Francisco, San Francisco, CA; 2) Dept. of Urology, University of California-San Francisco.

**Objectives:** Genetic male infertility has begun to explain a significant fraction of male infertility, despite our knowledge of only few identified genes. One way to improve our understanding of genetic causes of infertility is to identify candidate fertility genes and then to screen for gene mutations in a population of infertile patients. We have identified a candidate gene, *BOULE*, that is specifically expressed in human testes. Our goal is to try to study the function of this gene by screening for single nucleotide polymorphisms (SNPs) and mutations in this gene in a population of infertile patients. **Design:** Analysis of the coding region of *BOULE* gene by DNA sequencing and DHPLC analysis in a population of azoospermic and oligospermic men. **Methods:** DNA from a bank of infertile men (n=100 patients) was assessed for the integrity of *BOULE* gene. Primers were designed that surround and amplify each exon of the candidate gene by PCR. The PCR product for each exon was then used for detection of SNP or mutations by two techniques: DHPLC and sequence analysis. **Results:** There are total of 11 exons in this gene that encompass a 70kb genomic region. Initial screening for SNPs or mutations in exon 3 of this candidate male fertility gene in 50 infertile men has revealed 3 SNP sites in 2 azoospermic patients and 1 oligospermic patient. A systematic survey of all exons in more infertile patients and compared to a cohort of fertile patients is in progress. **Conclusions:** Identification of several SNPs in *BOULE* gene from a population of infertile men is encouraging, however, to make any link between those SNPs and male fertility, we need to compare these alterations to those found in normal fertile men and to examine RNA and protein expression of this candidate gene in the patients harboring those sequence variants.

**Characterization of FOXL2 mutations in patients affected with Blepharophimosis syndrome (BPES).** *V. Yellore<sup>1</sup>, N. Udar<sup>1</sup>, M. Chalukya<sup>1</sup>, S. Yelchits<sup>1</sup>, R. Silva-Garcia<sup>1</sup>, K. Dipples<sup>1</sup>, C. von Kap-herr<sup>2</sup>, M.J. Pettenati<sup>2</sup>, E. McCabe<sup>1</sup>, K. Small<sup>1</sup>.* 1) Department of Ophthalmology, Jules Stein Eye Inst and Department of Pediatrics, University of California, Los Angeles, CA; 2) . Department of Pediatrics, Section on Medical Genetics, Bowman Gray School of Medicine of Wake Forest University Medical Center, Winston-Salem, North Carolina 27157, USA.

Blepharophimosis syndrome (BPES) is a rare autosomal dominant disorder localized on human chromosome 3q23. This disorder is characterized by blepharophimosis (shortening of the palpebral fissures), ptosis, epicanthus inversus and telecanthus. In type I and type II BPES differ in the previous one being associated with female ovarian failures. Due to the defect in the palpebral fissure the patients tend to tilt their head backwards in order to see. There is also a high rate of sporadic mutations. The gene for this disease was cloned recently. The gene responsible for this disease is winged/forkhead transcription factor FOXL2. The level of proline and alanine are high in this protein. We have carried out mutation analysis on 30 individuals affected with this disease. We have identified mutations in 50% of these individuals. These mutations range for missense mutation to small deletion and duplication in the polyalanine and polyproline track. In patients with large deletions we have additional features like cardiac defects and mental retardations associated with the disease. Unidentified deletions and mutations in regulatory elements of the gene might account for some of the remaining patients.

**Genotype-phenotype correlations in methylmalonyl-CoA mutase deficiency.** *C. Cavicchi, A. Morrone, T. Bardelli, S. Funghini, M.A. Donati, G.M. Poggi, E. Pasquini, E. Zammarchi.* Dep. of Paediatrics, University of Florence, A.O. "A. Meyer", Florence, Italy.

Methylmalonyl-CoA mutase (MCM) deficiency is an autosomal recessive inborn error of metabolism, leading to methylmalonic aciduria (MMA). Biochemical cell studies delineate two phenotypic variants:  $\text{mut}^0$  with no detectable enzymatic activity and  $\text{mut}^-$  with a residual mutase activity. Clinical manifestations vary from benign persistent MMA to severe neonatal forms. The MCM enzyme comprises different functional domains: N-terminal domain involved in dimerization of two monomers, (b/a)<sub>8</sub> barrel domain with the CoA-binding site, C-terminal (b/a)<sub>5</sub> domain with the cobalamin-binding site. Three novel amino acid substitutions R108H, G215S and C560Y and a new small deletion c1758delA were identified in the MCM gene (MUT) of three Italian patients affected by neonatal severe form of  $\text{Mut}^0$  MMA. Both the R108H and G215S involve highly conserved residues of the barrel domain. The R108 is localised in the first b-strand of the barrel which lines the access channel for CoA ester substrates to the MCM active site. It can be hypothesized that the C560Y mutation, located in the linker region between barrel and C-terminal domain, alters the structural stability of MCM protein. The c1758delA leads to an early stop codon that destroys the B12 binding domain. The severe alteration of structure and/or function of MCM enzyme, caused by the above-mentioned mutations, demonstrates the deleterious nature of these defects, that can be correlated with the severe clinical phenotype. One patient with a mild form of neonatal  $\text{Mut}^0$  MMA was compound heterozygous for the new in frame deletion c1038-1040delTCT and for the G215S mutation. The c1038-1040delTCT results in deletion of a barely conserved leucine residue (346delL). Since the G215S was identified at homozygous level in one of the patients with severe MMA the c1038-1040delTCT can be correlated with the mild clinical phenotype. In addition two new (K212K and A499T) and two known (H532R, V671I) polymorphisms were identified. The financial support of Cometa-Italy is gratefully acknowledged.



**FGFR3 mutations associated with acanthosis nigricans increase keratinocyte survival via PI3-K/Akt signaling.**

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Acanthosis nigricans (AN) is a non-malignant hyperproliferative skin disorder that is often associated with metabolic states characterized by increased activity of growth factor signaling pathways (e.g. hyperinsulinemia or neoplasia). AN also occurs in patients who carry unique, activating FGFR3 mutations associated with the Crouzono-dermato-skeletal (A391E) and SADDAN (K650M) syndromes. Previously, we reported evidence that keratinocytes from patients with FGFR3 A391E or K650M mutations, in comparison to control keratinocytes, express increased amounts of the anti-apoptotic protein bcl-2 and are resistant to several different triggers that induce apoptosis. Based on this data we hypothesized that AN may result from prolonged survival of keratinocytes in the epidermis due to activation of signaling pathways that disrupt the normal process of differentiation and programmed cell death. In patients without FGFR3 mutations, AN is often associated with inappropriate activation of IGF-1 or EGF receptors by circulating growth factors. These receptors affect many signaling molecules including activation of PI3-K. PI3-K activation leads to phosphorylation of Akt which, in turn activates several different cellular survival signals. Using quantitative Western blots, we determined that, under growth factor deprived conditions, keratinocytes with FGFR3 A391E or K650M mutations have 3 to 7 times higher levels of phosphorylated Akt than control keratinocytes. Levels of the PI3-K inhibitor PTEN were not significantly different among mutant and control keratinocytes. Furthermore, treatment of FGFR3 A391E and K650M keratinocytes with the PI3-K inhibitors wortmannin or LY294002 restored levels of apoptosis caused by uv irradiation, cisplatin or beauvaricin to that of control keratinocytes. This data supports our hypothesis that AN results from increased survival of keratinocytes in the epidermal compartment and that activation of PI3-K/Akt by mutant FGFR3 receptors may play an important role in the pathogenesis of AN in Crouzono-dermato-skeletal syndrome and SADDAN syndrome.

**Molecular analysis and prenatal screening in spinal muscular atrophy in Singapore.** *P.S. Lai<sup>1</sup>, M.H. Liew<sup>1</sup>, S. Abbs<sup>2</sup>, S. Tay<sup>1</sup>, W.C. Yee<sup>3</sup>, A. Biswas<sup>4</sup>, S.C. Ng<sup>4</sup>, P.S. Low<sup>1</sup>.* 1) Dept Pediatrics, National Univ Singapore, Singapore, Singapore; 2) Genetics Centre, Guy's and St Thomas' Hospital Trust, London, UK; 3) National Neuroscience Institute, Singapore; 4) Dept Obstetrics & Gynaecology, Nat Univ Hospital, Singapore.

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by progressive anterior horn cell degeneration. There are three phenotypes based on clinical severity and onset age. All three types map to chromosome 5q13 and nearly 95% of patients display deletion of the SMN1 gene. The involvement of NAIP gene and possible modifier genes like H4F5 have also been implicated in some patients. We report a study on 31 SMA children of Asian ethnicity. 87% of patients show deletion of exon 7 of the telomeric SMN gene (SMNI), 74.2% showed deletion in exon 8, and 6.5 % showed NAIP gene deletion. Among the subtypes, homozygous deletion of exon 7 of SMNI was detected in 77.8%, 100% and 66.7% of type I, II and III patients respectively, homozygous deletion of exon 8 of SMNI was detected in 66.7%, 81.3% and 66.7% in the respective subtypes while NAIP was observed in 22.2% of type I patients only. This unusual pattern of homozygous deletion may be due to a different molecular basis for SMA in our population, such as presence of point mutations, conversion events and hybrid SMN gene. No SMNI or NAIP homozygous gene deletions have ever been found in our normal population, and it is clear that for affected patients, SMNI gene deletions would be diagnostic for SMA. We have also carried out prenatal diagnosis on a fetus conceived by intrauterine artificial insemination from an anonymous sperm donor. The family had a previous child also conceived by artificial insemination using sperms from the azoospermic father's brother who presented clinically with SMA type II. SMNI exons 7 and 8 were deleted in the affected child. Prenatal testing of the fetus demonstrated absence of the SMNI gene deletions. However, we were unable to determine in this particular analysis, if the fetus is a carrier. Nonetheless, since at least one SMNI gene copy is intact, this child should not be affected.

**Laminin is required to produce membrane instability in muscular dystrophy mediated by sarcoglycan loss.** *E.M. McNally*<sup>1</sup>, *K. Lavidos*<sup>2</sup>, *M. Hadhazy*<sup>1</sup>, *A.A. Hack*<sup>2</sup>. 1) Department of Medicine, The University of Chicago, Chicago, IL; 2) Department of Molecular Genetics Cell Biology, The University of Chicago, Chicago, IL.

The sarcoglycans, a group of transmembrane proteins in muscle and heart, are part of the dystrophin glycoprotein complex (DGC). Mutations in sarcoglycan genes cause cardiomyopathy and muscular dystrophy in humans and in mice by producing instability of the remaining sarcoglycans while leaving dystrophin intact. In contrast, mutations in dystrophin cause a reduction in not only the sarcoglycans, but also in additional DGC proteins such as the syntrophins and dystrobrevins. We used a genetic analysis to evaluate whether sarcoglycan is sufficient to mediate membrane instability and by generating mice lacking both dystrophin (*mdx*) and g-sarcoglycan. Mice lacking both g-sarcoglycan and dystrophin (*gdx*) had an identical phenotype to mice lacking only g-sarcoglycan with a similar degree of central nucleation and serum creatine kinase elevation. This demonstrates that sarcoglycan loss is sufficient to produce alterations in membrane permeability and the dystrophic process. In parallel experiments, we evaluated the role of the extracellular matrix protein laminin in the dystrophic process by producing mice mutant for both g-sarcoglycan and laminin-a2. Laminin-a2 is the major site of attachment to the extracellular matrix for the DGC. Mice mutant for both g-sarcoglycan and laminin-a2, (*gdy*), surprisingly displayed a severe dystrophic process yet had little evidence for disruption of membrane integrity. Little to no Evans Blue Dye uptake was seen and serum creatine kinase was only minimally elevated in (*gdy*) mice. These genetic analyses demonstrate that sarcoglycan loss is sufficient to produce the dystrophic process, and that laminin is required for disruption of membrane integrity and increased membrane permeability that is seen when sarcoglycan is absent.

**X-linked adrenoleukodystrophy associated with 10 novel ABCD1 gene mutations in 23 Portuguese families.** C.P. Guimaraes<sup>1,2</sup>, M. Lemos<sup>1,3</sup>, J.E. Azevedo<sup>1,2</sup>, M.C. Sá Miranda<sup>1,3</sup>. 1) Genetic Neurobiology, IBMC-UP, Oporto, Portugal; 2) ICBAS-UP, Oporto, Portugal; 3) Enzymology Department, IGMJM; Oporto, Portugal.

X-linked adrenoleukodystrophy (X-ALD) is a severe neurodegenerative disease. It has been associated with mutations in the ABCD1 gene, which encodes a peroxisomal half ATP-binding transporter (ALDP).

In order to characterize the molecular lesions underlying the biochemical defect, the mutational spectrum of the X-ALD Portuguese families was determined.

A RT-PCR based mutation screening was the preferred methodology. All the amplified products covering the entire coding region were analyzed by CSGE and only the altered fragments were sequenced. The results obtained show that the association of RT-PCR with CSGE analysis is highly efficient, since 100% of mutations are detected at the CSGE step. Using this strategy we have identified 14 missense, two nonsense mutations, three small deletions (two of them resulting in frameshift) and three splicing defects (of which two also allow the production of normal mRNA molecules). Additionally, two polymorphisms were found. The identification of 10 new mutations and 1 new polymorphism adds to the large number of genetic alterations already described as being associated with X-ALD.

Immunoblotting studies were also performed in patient's fibroblasts. Normal levels of ALDP were found in three patients and decreased in all the others affected with missense mutations. In the two splicing cases where we observed the presence of non-mutated ALDP, the levels of this protein were quantified. In patients with either nonsense, frameshift mutations or with a double mutated gene (with two missense) ALDP was not detectable.

The molecular analysis of X-ALD gene still remains of extreme importance in the pre-natal diagnosis, carrier detection and in the early identification of at risk males. The characterization of mutations in X-ALD would also be important for the elucidation of X-ALD pathogenic mechanism.

(C.G. is financially supported by FCT SFRH/BD/1445/2000).

**The contribution of genotypes at the SAA1 locus to amyloidosis and disease severity in patients with Familial Mediterranean fever.** *R. Gershoni-Baruch*<sup>1,3</sup>, *R. Brik*<sup>2,3</sup>, *M. Shinawi*<sup>1</sup>, *T. Paperna*<sup>1</sup>, *A. Livneh*<sup>4</sup>. 1) Institute of Human Genetics, Rambam Medical Center, Haifa, Israel; 2) Pediatric Rheumatology Service, Rambam Medical Center; 3) The Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; 4) Heller Institute, Tel-Hashomer, Israel.

Familial Mediterranean fever is an autosomal recessive disease characterized by recurring attacks of fever and serositis. Amyloidosis, leading to renal failure, is the most severe manifestation. Mutations in the pyrin/marenostrin (MEFV) gene have been identified in the majority of patients. The wide clinical variability of the disease has been partly attributed to MEFV allelic heterogeneity with the M694V mutation and particularly the M694V/M694V genotype being associated with a severe phenotype and amyloidosis. Since patients with identical mutations vary in their clinical manifestations especially as regards the development of amyloidosis, a role for additional genetic and/or environmental modifiers has been proposed. Recently, polymorphisms at the SAA1 (serum amyloid A1) locus, or rather the SAA1a/a genotype, were found to influence susceptibility to renal amyloidosis. In this study we evaluated the contribution of genotypes at both the MEFV and the SAA1 loci to disease severity and amyloidosis. DNA samples from 220 FMF patients (42 of them with amyloidosis) in whom two mutant FMF alleles have been identified, were further analyzed for genotypes at the SAA1 locus. Disease severity was calculated according to Tel-Hashomer severity score. Of the 42 patients with amyloidosis 33 (78%) were M694V homozygotes, while the rest were compound heterozygotes for either the M694V or the complex V726A-148Q allele. Fourteen of them (33%) had the SAA1a/a genotype in comparison to only 17/178 (10%) of the pt's without amyloidosis. No correlation was found between disease severity and the presence of the SAA1a/a genotype. The results of this study agree with the observation that the SAA1 locus plays a key role in conferring genetic susceptibility to amyloidosis. Otherwise, polymorphisms at the SAA1 locus were not found to be associated with disease severity.

**Targeted disruption of the Diamond-Blackfan anemia homologue *Rps19* in mice.** *H. Matsson*<sup>1</sup>, *D. Drapchinskai*<sup>1</sup>, *E. Davey*<sup>1</sup>, *E. Forsberg*<sup>2</sup>, *P. Leveen*<sup>3</sup>, *S. Karlsson*<sup>3</sup>, *N. Dahl*<sup>1</sup>. 1) Genetics and Pathology, The Rudbeck Laboratory, Uppsala University, Sweden; 2) Medical Biochemistry and Microbiology, BMC, Uppsala, Sweden; 3) Gene Therapy Center, WNC, University of Lund, Sweden.

Diamond-Blackfan anemia (DBA) is a rare bone marrow failure syndrome characterized by specific decrease or absence of erythroid precursors. Additional anomalies are present in 20-30% of cases including short stature, limb malformations and heart defects. Patients present with a variable expression of the disease and the overall mortality rate is approximately 30%. The disease may be inherited as a dominant trait but most cases are sporadic. The molecular mechanisms behind DBA are unknown. However, the gene encoding ribosomal protein S19, *RPS19*, is mutated in 25% of patients. The *RPS19* gene consists of six exons of which five encode a 16-kDa protein located in the small ribosomal subunit. Diamond-Blackfan anemia is the first proven example of a disease caused by mutations in a gene encoding a ribosomal protein, suggesting an extra-ribosomal role of the S19 protein. Evidence for additional functions of ribosomal proteins have previously been gathered from studies of *D. melanogaster* and *C. elegans* models. In human, a homodimer of *RPS19* is believed to act as a chemotactic factor involved in removal of apoptotic cells. A mouse model with one *Rps19* allele deleted for exon 1-5 was created in order to gain new insight into the functions of the ribosomal protein S19. We show that mice with targeted disruption of one *Rps19* allele are viable. Male mice are sterile and associated with a reduced testicular volume whereas female mice show reduced fertility. Reduced fertility is not reported in patients with DBA and our findings suggest an extra-ribosomal function for *Rps19* in mouse gonadal development.

**MEFV mutant alleles sustain a differential role in the clinical profile of familial Mediterranean fever. M.**

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Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurring attacks of fever and serositis. Amyloidosis, leading to renal failure, is the most severe manifestation. Five sequence alterations (M694V, V726A, M680I, M694I and E148Q) in the MEFV gene account for the majority of FMF chromosome. The wide clinical variability of the disease has been attributed, in part, to MEFV allelic heterogeneity. Homozygotes for the M694V mutation have a more severe form of the disease and more frequently demonstrate articular and renal manifestations. Mutations E148Q and V726A have reduced penetrance. The clinical features, associated with the M680I and the complex V726A-E148Q allele, are not well defined. The aim of this study is to further characterize the phenotypic profile associated with the major MEFV mutations, particularly M680I, M694I and the complex V726A-E148Q alleles, predominant in our FMF patients of Arab origin. We investigated 230 FMF patients (135 Jewish and 95 Arabs) in whom both FMF alleles have been identified and found that different mutations and genotypes are distinguished by a differential clinical profile and penetrance. Homozygotes for mutations at the 694 locus (M694I and M694V) are frequently severely affected. Homozygotes for the complex V726A-E148Q allele are at least as severely affected and often endure renal amyloidosis. The complex V726A-E148Q and the M694V alleles are associated with the most severe form of the disease. Compound heterozygotes for the complex V726A-E148Q allele in combination with either the E148Q, the V726A or the M680I allele are more severely affected than compound heterozygotes for the M694V allele with either of these alleles. The morbidity associated with the complex V726A-E148Q allele by far outweighs that associated with the V726A allele, bearing evidence to the fact that the E148Q mutation is not a benign polymorphism.

**Genetic Polymorphism of Aldehyde dehydrogenase in Chinese: Sex, Age, Geography, Culture and Genotypes of ALDH2.** *H-R. Luo, Y-P. Zhang.* Lab Cellular & Molec Evol, Kunming Inst Zoology, Kunming, Yunnan 650223, People's Republic of China.

The genetic polymorphisms of the aldehyde dehydrogenase-2 (ALDH2) locus have been considered a potential explanation for individual differences in drinking behavior and those individuals with the defected, atypical ALDH2<sup>2</sup> alleles are alcohol flushers because of the acetaldehyde can't be metabolized in time. In order to understand the relation between the age, sex, geography and the genotypes of ALDH2, we have invested the ALDH2 genotypes in total six hundred and thirty-eight unrelated healthy Chinese samples from 7 Chinese geographic populations with different sex and age in current assay. The frequency for the atypical ALDH2<sup>2</sup> allele in the total samples was 17.71%. The frequencies of the ALDH2<sup>1</sup>/ALDH2<sup>1</sup>, ALDH2<sup>1</sup>/ALDH2<sup>2</sup> and ALDH2<sup>2</sup>/ALDH2<sup>2</sup> genotypes in the 638 subjects were 68.65% (438/638), 27.27% (174/638) and 4.08% (26/638), respectively. The frequencies of the individuals with the atypical ALDH2<sup>2</sup> allele was ranged from 22.00% (Wuhan Han) to 54.35% (Guangdong Han) in different Chinese geographic groups, which was similar to that of other reported Asian populations (28%-56%). The individuals from Guangdong Han population had significantly higher frequencies of the atypical ALDH2<sup>2</sup> alleles (30.98%) than the other geographic populations had (12.00%-20.00%)( $p < 0.01$ ), which suggested that there were some differences in the atypical allele frequency in different geographic populations. The different sex and age had little effect on the frequencies of ALDH2<sup>2</sup> allele ( $p > 0.05$ ). The low alcohol consumption in Chinese women could be explained by culture, but not genotypes of ALDH2. The probable reason for the no difference between the three age groups (Infant, Young and Old) was that the individuals with the atypical alleles could discount the alcohol intake, and consequently the defected alleles did not affect the survival of the individuals. This was the first report about age-related ALDH2 genotypes, so the results of this assay could provide some information for the further studies of ALDH2 genotypes.



**Association between c-erbB-2 codon 655 polymorphism and NF1.** *L. Fishbein, S.A.M. Thomson, B. Eady, M.R. Wallace.* Molecular Genetics and Pediatric Genetics, Univ. of Florida, Gainesville, FL.

Neurofibromatosis 1 (NF1) is an autosomal dominant disorder characterized by overgrowth of neural crest derived tissues, such as neurofibromas. There is variable expressivity and it is thought that other genes likely modify NF1 phenotype. The proto-oncogene c-erbB-2 (or HER2/neu) encodes a tyrosine kinase receptor. A member of the epidermal growth factor receptor family, c-erbB-2 is thought to control cellular functions such as cell proliferation. After dimerization with other erbB family members and activation, c-erbB-2 transmits a signal downstream to cytoplasmic targets, including the MAPK pathway, also downstream of NF1. ErbB proteins have roles in neural crest proliferation and/or have been implicated in neurofibroma development. The c-erbB-2 gene is frequently amplified or over-expressed in breast cancer and a number of adenocarcinomas. Animal models of neurofibromas have a specific activating c-erbB-2 mutation, although no human tumors have been reported with this mutation. An Ile/Val polymorphism exists at codon 655 in the transmembrane domain near the mutation. It is not known if this polymorphism results in different tyrosine kinase activities. However, there is an association of the Val allele with an increased risk for breast cancer. The role that the c-erbB-2 gene product plays in cell proliferation, as well as the critical function of the transmembrane domain in the receptor activation, make this polymorphism a strong candidate for a modifier gene for NF1. As an initial analysis, we examined the association between the c-erbB-2 codon 655 alleles and NF1. Our study has found that the Val allele was less prevalent ( $p=0.009$ ) among NF1 patients ( $n=184$ ) than among our control populations ( $n=274$  combined). In fact, the number of NF1 patients with a homozygous Val/Val genotype is significantly lower ( $p=0.044$ ) than that found in our control populations. Despite this, the number of heterozygous Ile/Val and Ile/Ile genotypes is not significantly different in the NF1 population. This may suggest that there is a selective mortality associated with the combination of an NF1 mutation and a c-erbB-2 homozygous Val/Val genotype.

**Homologous sequences between *CHST5* and *CHST6*, which occur gene conversion and cause macular corneal dystrophy type II.** *T. Akama*<sup>1</sup>, *A. Tanigami*<sup>2</sup>, *M.N. Fukuda*<sup>1</sup>. 1) Glycobiology Program, The Burnham Inst, La Jolla, CA; 2) Otsuka GEN Research Institute, Tokushima, Japan.

*CHST6* (carbohydrate sulfotransferase 6) is located next to *CHST5* with the same orientation on Chr.16q22. These two genes are highly homologous to each other not only in the coding region but also in the upstream and downstream sequences, suggesting that *CHST5* and *CHST6* were created by gene duplication during evolution<sup>1</sup>. This hypothesis is supported by the fact that mouse genome has only one orthologue (*Chst5*) corresponding to *CHST5* and *CHST6*<sup>2</sup>. Unequal cross-over and gene conversion between *CHST6* and *CHST5* cause a lack of enzymatic activity of corneal GlcNAc 6-O sulfotransferase, encoded by *CHST6*, in corneal cells and result in a recessive hereditary eye disease, macular corneal dystrophy (MCD). By sequencing of a BAC clone that contains *CHST5* and *CHST6*, and by collecting sequences from human genome database, we assembled a large contig covering *CHST5* and *CHST6*. We also identified junction sequences of a gene conversion found on *CHST6* of MCD patient's genome. A 3 kbp converted region was located on 4.2 kbp upstream of *CHST6* exon 1 and each of both flanking sequences of the donor (*CHST5*) and acceptor (*CHST6*) were highly homologous (more than 90 %) to each other. Chi-like sequences were also found in the flanking sequences of the converted region. The upstream region of *CHST6*, which is lost in MCD type II, may contain corneal epithelium-specific enhancer because MCD type II patient lacks *CHST6* expression in the corneal epithelium but not in the other tissues such as cartilage.

1. Akama, T.O., et. al., *Nature Genet.* **26**, 237-241 (2000)
2. Akama, T.O., et. al., *J. Biol. Chem.* **276**, 16271-16278 (2001)

This work is supported by NIH CA71932.

**Regulation of expression of the *JAGGED1* gene responsible for Alagille syndrome.** *M. Meunier-Rotival<sup>1</sup>, L. Luron<sup>1</sup>, C. Crosnier<sup>1</sup>, N. Raynaud<sup>1</sup>, C. Driancourt<sup>1</sup>, J. Boyer<sup>1</sup>, M. Hadchouel<sup>1,2</sup>.* 1) Genetique et maladies du foie de l'enfant, INSERM U347, Kremlin-Bicetre, France; 2) Service Hepatologie pediatrique, Hopital de Bicetre, France.

Heterozygous mutations in *JAGGED1*, encoding a ligand for Notch receptors, cause Alagille syndrome (MIM 118450), a polymalformative disorder affecting mainly the liver, heart, skeleton, eye and face. Minor features involving kidney, arteries, ear, and limb have also been described. Haploinsufficiency could be invoked due to cytogenetically visible deletions in 20p12, or mutations in the very 5'-translated part of the gene. The sites of *JAGGED1* transcription during human development are correlated with tissues affected by the major and minor features of the syndrome. The Notch signaling pathway is known to be very sensitive to gene dosage. To gain insight into the regulation of *JAGGED1* expression, we performed transient transfections with the Dual Luciferase Reporter Assay system in Cos cells. Five overlapping fragments corresponding to the 5'-flanking sequence (ranging from -239 to -2760 bp) were tested. A high luciferase activity was detected with the fragment spanning 500 bp upstream of the mRNA start, suggesting the presence of positive regulatory elements. Computational analyses indicated various binding sites for transcription factors such as Sp1, AP1, AP2, and GATA, in this region. These factors mostly correspond to ubiquitous factors (except for GATA), and could be involved in the basic activity of the promoter. Given that *JAGGED1* is mainly expressed in the cardiovascular system, experiments with other cell lines including endothelial cells will be performed to test the tissue-specificity of *JAGGED1* expression. meunier@infobiogen.fr.

**Genetic mechanisms of puberty-onset amelioration of hemophilia B Leyden: animal models, and critical roles of ASE and growth hormone.** A. S. Kurachi, J. Huo, A. Ameri, H. Atoda, K. Kurachi. Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

Hemophilia B Leyden is characterized by its unique puberty onset amelioration of bleeding. This phenotype is caused by specific single-nucleotide mutations clustered in a small region (LSR) of the 5' UTR of human factor IX (hFIX) gene. Recently we determined the age-regulatory mechanisms of the hFIX gene, identifying two critical genetic elements ASE and AIE, which are essential for age-dependent stable and increasing expression patterns of the gene, respectively (Science 1999;285:739-743). These findings enabled us to construct transgenic mouse models, robustly recapitulating the Leyden phenotype. Both male and female transgenic mice carrying -802(-20A)FIXm1, a mutant hFIX minigene which contains ASE and a Leyden mutation at nt -20 (T to A change), but not animals carrying a hFIX Leyden minigene, -416(-20A)FIXm1 which lacks ASE, fully recapitulated the unique Leyden characteristics (puberty-onset induction of hFIX gene expression). Hypophysectomy and growth hormone (GH) supplementation performed with these transgenic animals showed that the GH signal transduction pathway, but not that of androgen, is directly responsible for the amelioration, supporting that both male and female animals can have the hFIX Leyden phenotype. Animal models of other Leyden mutations (nt +13) as well as a null-amelioration mutation at nt -26 (Brandenburg phenotype) were also successfully constructed. Together, these observations indicate that the mechanisms of puberty-onset amelioration of hemophilia B Leyden involve a unique set of gene expression switches occurring through the puberty period. After years of elusiveness, the basic genetic mechanisms of hemophilia B Leyden have finally been established. Such mechanisms are also being implicated in subsequent age-associated regulation not only of the hFIX gene but also many other genes.

**Phenotypic and genetic heterogeneity of unexplained neonatal/early infantile respiratory distress in Reunion**

**Island : SP-B deficiency and alveolar proteinosis.** *M. Tredano*<sup>1</sup>, *M. Griese*<sup>2</sup>, *J. de Blic*<sup>1</sup>, *F. Capron*<sup>1</sup>, *E. Mallet*<sup>3</sup>, *C. Houdayer*<sup>1</sup>, *J-L. Alessandri*<sup>3</sup>, *D. Feldmann*<sup>1</sup>, *R. Couderc*<sup>1</sup>, *J. Elion*<sup>1</sup>, *M. Bahuaud*<sup>1</sup>. 1) Biochimie et Biologie Moléculaire (M.T., C.H., D.F., R.C., M.B.), Hôpital Trousseau ; Pneumologie et Allergologie Pédiatriques (J.B.), Hôpital des Enfants-Malades, Paris ; Anatomie et Cytologie Pathologiques (F.C.), Hôpital Bécclère, Clamart, France; 2) Dr. von Haunerschen Kinderspital, München, FRG; 3) Néonatalogie (E.M.), Hôpital Sud Réunion ; Pédiatrie (J-L.A.), Hôpital Félix-Guyon, Ile de La Réunion, France.

Two main discrete entities with primary/unexplained hereditary or acquired lung disease leading to respiratory distress (RD) are characterized by storage of periodic acid Schiff-positive material within the alveoli : i, SP-B deficiency, with an essentially proteinaceous storage and rapid fatal outcome in the neonate, linked to *SFTPB* gene (MIM 1786640); ii, alveolar proteinosis (AP), clinically heterogeneous (age at onset/severity), with storage of a mixed, protein/lipid material. Akin to SP-B deficiency, primitive congenital/severe infantile AP is regarded as Mendelian autosomal recessive. Unexplained RD is unusually common in the white settler sub-population of Reunion Island, suggesting founder effect. However, no deleterious mutation or disease locus has been identified. We report a consanguineous male Caucasian infant from Reunion Island who had RD in his neonate and died at age 1 month. There was no immunodetectable SP-B in postmortem lung tissue. DNA analysis unveiled a novel homozygous mutation in *SFTPB* exon 2 (496delG). This mutation, or any other *SFTPB* lesion, was not found in the DNAs of nine patients with early infantile AP diagnosed by lung ultrastructural study, or two patients with neonatal RD, all from Reunion Island. In addition, some of these patients stained positive for SP-B in their bronchoalveolar lavage (BAL) fluid and the ones diagnosed with AP are maintained by repeated therapeutic BAL. Altogether, these results highlight the phenotypic and genetic heterogeneity of neonatal RD in Reunion Island, only a fraction of which could be linked to *SFTPB*.

**Analysis of mannose binding lectin (MBL) haplotypes in a large cohort of cystic fibrosis patients.** *J.S. Zielenski<sup>1</sup>, L. Pereira<sup>2</sup>, L. Culpi<sup>2</sup>, S. Raskin<sup>2</sup>, L.C. Tsui<sup>1</sup>*. 1) Department of Genetics, Hospital for Sick Children, Toronto, ON., Ontario, Canada; 2) Department of Genetics, Universidade Federal do Parana, Parana, Brazil.

Cystic fibrosis (CF) is an autosomal, recessive disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. The considerable phenotypic variability of CF is attributed to CFTR genotype as well as secondary genetic and environmental factors. The mannose binding lectin (MBL), a collectin that is important for innate immunity to infection, has been found in previous studies to have an effect on CF pulmonary disease, including infection status, the age-adjusted, percent predicted forced expiratory volume in one second (%predFEV1) and forced vital capacity (FVC). Several MBL variants have been associated with low levels of MBL protein in serum and, therefore, a compromised efficiency of the innate response. We have analyzed the haplotypes of the sequence variations of the gene at nucleotide position -221 and codons 52, 54 and 57 among 480 CF patients, with respect to a series of clinical parameters, including age of onset of *Pseudomonas aeruginosa* (Pa) and *Burkholderia cepacia* (Bc) infection, the infection status for Pa, Bc, *Haemophilus influenza*, *Staphylococcus aureus* and *Klebsiella* species (particularly at ages of 6, 12 and 24 months), as well as and FEV1 FVC. The result showed that the low MBL secretion haplotypes were significantly associated with earlier age of Pa infection ( $P < 0.05$ ). Also, a trend was detected between the low secretion haplotypes and poor pulmonary function as measured by infection status, pulmonary function and survival. There was no significant difference between patients homozygous for most common CFTR mutation, deltaF508, and other severe CFTR genotypes. Therefore, MBL may be considered as a modifier gene for CF pulmonary disease.

**The effect of cellular and viral splicing factors on the level of normal CFTR RNA.** *M. Nissim-Rafinia, B. Kerem.*  
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Variable levels of aberrantly spliced CFTR transcripts, carrying splicing mutations, were shown to correlate with variable CF severity. We suggested that the mechanism underlying this variability involve splicing factors known to regulate alternative splicing. We focus on two mutations causing alternative splicing of the CFTR RNA: the 3849+10kb C®T which can lead to cryptic exon inclusion and the IVS8-5T (5T) which can lead to exon 9 skipping. We constructed minigenes containing these mutations and studied the effect of splicing factors on the splicing pattern of these minigenes. Overexpression of the cellular factors ASF/SF2 and hnRNP A1, and the viral factor E4-ORF6 promoted exon skipping of RNA transcribed from the minigenes. Thus, in the 5T minigene, it led to a substantial decrease in the level of correctly spliced RNA, while in the 3849+10kb C®T minigene it led to the generation of correctly spliced RNA that was not found without this overexpression. The viral factor, E4-ORF3 led to the antagonistic effect, thus led to a substantial increase of the correct transcripts from the 5T minigene.

We further studied the modulation effect of the splicing factors on native CFTR alleles carrying the 3849+10kb C®T mutation. For this we established a nasal epithelial cell line, 091398k, from a nasal polyp of a CF patient carrying the mutation. 21% of the transcripts in 091398k cells are aberrantly spliced. Overexpression of the SR proteins ASF/SF2, SC35 and SRp20, hnRNP A1 and E4-ORF6 into these cells promoted exon skipping, and led to a decrease in the level of aberrantly spliced transcripts. The most significant effect was achieved with ASF/SF2, which led to almost a complete abolishment of the aberrantly spliced transcripts. The viral factor E4-ORF3 and the non-essential cellular factor Htra2-b1 promoted exon inclusion and led to a significant increase in the level of aberrantly spliced transcripts (up to 80%). Thus, cellular and viral splicing factors might play an important role in the mechanism underlying phenotypic variability in CF and other genetic diseases, caused by splicing mutations.

**Constraint on intron size as a mutational mechanism in Rothmund-Thomson Syndrome.** *S.E. Plon<sup>1</sup>, L.L. Wang<sup>1</sup>, A. Gannavarapu<sup>1</sup>, K.C. Worley<sup>2</sup>*. 1) Dept Pediatrics, Baylor Col Medicine, Houston, TX; 2) Human Genome Sequencing Center, Baylor Col Medicine, Houston, TX.

Rothmund-Thomson Syndrome(RTS) is an autosomal recessive disorder characterized by poikiloderma, skeletal anomalies, cataracts and predisposition to osteosarcoma. *RECQL4* mutations have been reported in four kindreds. We are sequencing the entire *RECQL4* gene in a cohort of 34 RTS probands. The unusual *RECQL4* gene structure contains 21 exons in six kb of DNA, with 13 introns less than 100 bp in length. One RTS proband (FCP102) with severe rash and OS from a consanguineous Mexican family is homozygous for an 11 bp deletion in intron 8 (g2746del11), which was not found in 100 control subjects including 33 Hispanics. The deleted sequence, which is flanked by an 8 bp direct repeat, includes no consensus splice or branch sites but reduces the intron size to 66 bp. We hypothesized that this deletion creates an intron of insufficient size to splice correctly. RT-PCR of lymphoblast and fibroblast RNA from FCP102 revealed only abnormal splice products that retain intron 8, resulting in a frameshift and premature stop. We subcloned genomic DNA between exons 5 and 10 from control and FCP102 into a mammalian expression vector. RT-PCR of cytoplasmic RNA from transfected NIH3T3 cells showed that the deleted intron is not spliced. Thus, this intronic deletion disrupts the *RECQL4* gene. To prove this results from a constraint on intron size, site directed mutagenesis was used to replace the deletion with 11 unrelated bases. Transfection of this construct demonstrated that the replaced intron is correctly spliced. We identified a second RTS proband with a 24 bp deletion in intron 13 (g3712del24) resulting in a 54 bp intron. RT-PCR also showed abnormal RNA products consistent with exon skipping. Deletions in small introns will be missed by most exon-by-exon mutation scanning or sequence protocols. Analysis of mRNA sequences mapped to the human genome sequence reveals approximately 17% of genes with at least one intron less than 100 bp. Thus, complete mutational analysis should include assaying small introns for deletions.



**Characterization of the splicing mutation in *IKBKAP* that causes Familial Dysautonomia.** *S.A. Slaugenhaupt<sup>1</sup>, M. Leyne<sup>1</sup>, J. Mull<sup>1</sup>, S.P. Gill<sup>1</sup>, M.P. Cuajungco<sup>1</sup>, J.F. Gusella<sup>1</sup>, N. Dormand<sup>2</sup>, R. Reed<sup>2</sup>.* 1) Harvard Inst Human Genetics, Mass General Hosp, Boston, MA; 2) Dept of Cell Biology, Harvard Medical School, Boston, MA.

Recently we reported that two mutations in the gene *IKBKAP* cause Familial Dysautonomia (FD). FD is an autosomal recessive disorder present in 1 in 3,600 live births in the Ashkenazi Jewish population. *IKBKAP*, which is homologous to yeast Elp1 and believed to play a role in transcriptional elongation, harbors two mutations that are responsible for FD. The major mutation accounts for >99.5% of all disease chromosomes and is located at base pair 6 of intron 20. The result of this mutation is an apparent decrease in splicing efficiency that results in variable skipping of exon 20. Interestingly, despite the fact that FD is a recessive disease, normal mRNA and protein are still expressed in all patient cells suggesting that this mutation weakens but does not completely inactivate the splice donor site. The second mutation is a missense (R696P) in exon 19 that has been seen in only four FD patients that are heterozygous for the major splicing mutation. New assays designed to detect only the wild-type or mutant message show that lymphoblasts and fibroblasts express primarily wild-type mRNA, while FD brain expresses primarily mutant. We are currently examining the expression of *IKBKAP* in a variety of FD tissues. In addition, we are studying the effect of various culture conditions to determine if we can influence the splicing ability of FD cells. In order to investigate the role of the major mutation in defective splicing and to determine at which step the splicing process is hindered, we have also created a minigene construct containing exons 20 and 21 and 180 bp's of flanking intron 20 sequence. This model will enable us to determine the exact effect of the FD mutation, and provide a system to study the effect of various splicing factors on the mutant splicing pattern. Since all patients show wild type message and apparently normal protein, the prevention of exon 20 skipping by upregulating normal *IKBKAP* mRNA represents an exciting potential target for future therapy of FD.

**Predicted expression of *PLP1* splicing mutations in Pelizaeus-Merzbacher disease.** *S.R. Svojanovsky*<sup>1</sup>, *G.M. Hobson*<sup>2</sup>, *K. Sperle*<sup>2</sup>, *E.A. Sijtermans*<sup>3</sup>, *J.Y. Garbern*<sup>4</sup>, *P.K. Rogan*<sup>1</sup>. 1) Children's Mercy Hospital, Kansas City, MO; 2) A.I. duPont Hospital for Children, Wilmington, DE; 3) Univ Med Ctr, Nijmegen, Netherlands; 4) Wayne State Univ Sch Med, Detroit, MI.

Mutations in the *PLP1* gene cause dysmyelinating phenotypes in Pelizaeus-Merzbacher disease. The DM20 splice form of this gene, which utilizes an alternative donor site in exon 3, can partially compensate for *PLP1* in patients with mutations in the latter half of this exon. We examined the severity of splicing mutations that affect expression of the *PLP1* and/or DM20 transcripts. *PLP1* variants suspected or documented to alter splicing (n=28) were evaluated by individual information ( $R_i$ ) analysis (Hum. Mut. 12:152,1998) and compared with available mRNA expression data. Reduced binding affinity to the natural splice site, i.e. decreasing but not abolishing normal *PLP1* mRNA, was predicted by information analysis for IVS2+6T>C [-2.6 x], G453T [-9.8 X], G453C [-16 X], IVS3+4A>G [-5.7 X], A620G [-2 X], and IVS6+3G>T [-17 X]. Leaky expression of *PLP1* mRNA was found in IVS3+4A>G, but not for G453T or IVS6+3G>T, which are predicted to reduce mRNA levels to a greater extent, possibly below the detection limit. *PLP1* splicing was predicted to be inactivated [at least  $\sim 100$  X] for IVS3+2T>C, IVS5-1G>A, IVS6+1G>A, and IVS6+2T>C, which was confirmed for IVS3+2T>C. Cryptic splicing and inactivation of the natural site was predicted and confirmed for IVS2-2A>G, IVS3-2A>G, IVS3-1G>T, IVS5+2T>C, but not for IVS5+2T>G, where the activated cryptic site in IVS5+2T>C may not be used. T116K strengthens the DM20 splice site 4-fold to 7.4 bits and eliminates use of the weaker, 6.8 bit exon 3 donor. The following variants were not expected to alter *PLP1* or DM20 mRNA: T2C, G3A, G3del, A168G, C384G, C409G, A441T, IVS3+28-+46del, IVS4+28C>G; normal splicing has been verified for G3del and IVS4+28C>G. The observed *PLP1* and DM20 splicing patterns corresponded to predictions of information theory-based splice site analyses in nearly all instances studied.

**The Molecular Basis of Glycogen Storage Disease Type 1a: Structural and Functional Analysis of Mutations in Glucose-6-Phosphatase.** *J.J. Shieh, M. Terzioglu, C.J. Pan, L.Y. Chen, H. Hirawa, J.Y. Chou.* HDB/NICHD, National Institutes of Health, Bethesda, MD.

Glycogen storage disease type 1a (GSD-1a) is caused by the deficiency in glucose-6-phosphatase (G6Pase), a nine-helical endoplasmic reticulum transmembrane protein required for maintenance of glucose homeostasis. To date, 75 G6Pase mutations have been identified in GSD-1a patients, including 48 mutations result in single amino acid substitution. However, only 19 such mutations have been functionally characterized. Here, we report the results of structural and functional studies of all 48 missense and the delF327 mutations. While 31 missense and delF327 mutation abolish G6Pase activity, 18 missense mutations retained the activity ranging from 0.7 to 24% of wild-type enzyme. Immunoblot analysis shows that a large number of those mutants support the synthesis of lower amounts of G6Pase protein than that of the wild-type construct. This suggests the structural requirement for an active G6Pase is very rigid and disruption of the structural integrity of the enzyme destabilizes the protein. This mutational information also explains why the islet-specific G6Pase catalytic subunit-related protein is devoid of enzyme activity. Taken together, we have generated a data-base of G6Pase mutation that cause GSD-1a and elucidated the structural requirements for the stability and enzymatic activity of G6Pase.

**Distribution of Usherin in humans and its effects on reproduction in people with Usher Syndrome Type II.** *N.A. Pearsall<sup>1</sup>, G. Bhattacharya<sup>1</sup>, D. Cosgrove<sup>1</sup>, J.L. Wisecarver<sup>2</sup>, W.J. Kimberling<sup>1</sup>*. 1) Genetics Department, Boys Town National Research Hospital, Omaha, NE; 2) Pathology Department, University of Nebraska Medical Center, Omaha, NE.

Usher Syndrome is an autosomal recessive disease that results in varying degrees of hearing loss and retinitis pigmentosa. Three types of Usher Syndrome (I, II, and III) have been identified clinically and are distinguished by severity and progression of hearing loss along with the presence or absence of vestibular dysfunction. Usher Syndrome type II is the most common of the three types and has been localized to three different chromosomes 1q41, 3p, and 5q, corresponding to Usher type 2A, 2B, and 2C respectively. Usherin is a basement membrane protein encoded by the USH2A gene. Expression of usherin has been localized to the basement membranes of several tissues but it is not ubiquitous.

Immunohistochemistry detected usherin in the following human tissues: retina, small and large intestine, pancreas, bladder, prostate, thyroid, esophagus, thymus, salivary glands, placenta, ovary, oviduct, and fallopian tube. Usherin was not detected in many other tissues such as heart, lung, liver, kidney, and brain. This distribution is consistent with the usherin distribution seen in the mouse. Immunoperoxidase staining of the placenta or salivary glands may provide an effective and minimally invasive way to diagnose a person with Usher IIa.

The presence of usherin in the reproductive tissues prompted the investigation of reproductive patterns among those with Usher II. Evaluation of pregnancy and birth rates of those with Usher II demonstrates no statistical difference from those not affected with Usher II. Similar data is being collected to determine if people with Usher I have comparable reproductive patterns. Although normal usherin expression is essential for proper development and function of both the inner ear and retina, it does not seem necessary for reproduction in people with Usher II. Nevertheless, evolutionary conservation of expression in tissues unaffected in USH2A raises interesting issues regarding the source of evolutionary pressures.

**PFGE analysis of 4qter-10qter interchromosomal exchanges in Italian families with Facioscapulohumeral muscular dystrophy (FSHD).** *L. Felicetti*<sup>1,2</sup>, *G. Galluzzi*<sup>1,2</sup>, *L. Colantoni*<sup>1,2</sup>, *M. Rossi*<sup>1,2</sup>, *B. Merico*<sup>2</sup>, *F. Mangiola*<sup>1</sup>, *P. Tonali*<sup>2</sup>, *E. Ricci*<sup>2</sup>. 1) Ctr Neuromuscular Diseases, UILDM, Rome, Italy; 2) Institute of Neurology, Catholic University, Rome, Italy.

In 95% of FSHD patients, molecular diagnosis is based on the detection by probe p13E-11 of EcoRI, BlnI-resistant fragments of 10 to 35 kb, shorter than those found in normal individuals (35-300 kb). The size reduction is due to a deletion of a variable number of KpnI repeats at 4q35 region. 4q-10q interchromosomal exchanges occur both in normal and FSHD subjects, resulting in the reshuffling of 4q-type BlnI-resistant and 10q-type BlnI-sensitive repeats from one chromosome to the other. We analyzed the segregation of BlnI-resistant and BlnI-sensitive alleles in 55 FSHD Italian families for a total of 230 individuals (116 affected, 70 unaffected and 44 spouses). DNA extraction and subsequent restriction steps with EcoRI, BlnI and Tru9I were performed directly in agarose blocks. After separation by Pulsed Field Gel Electrophoresis (PFGE), the alleles were identified with p13E-11 and KpnI cloned sequences as probes. We observed different types of exchanges: total 4q to 10q transfers resulting in trisomy and tetrasomy; partial 10q to 4q transfers leading to monosomy and nullisomy; complex rearrangements such as multiple translocations, even in subjects with p13E-11 standard allele configuration. In addition, we detected two patients carrying 10q-4q translocations involving the short fragments associated with FSHD. Among 8 sporadic cases carrying a de novo p13E-11 small fragment (BlnI-resistant), we found four patients, with no evidence of somatic mosaicism, with one or both parents showing various types of rearrangements (trisomy, monosomy, etc.). The remaining four were somatic mosaics, all carrying 4q-10q translocations. Our results show the high frequency of 4qter-10qter interchromosomal exchanges in FSHD families and confirm that the instability of subtelomeric regions can play a role in the molecular mechanism of the disease. Telethon Italy grant n.1296.

**Frequency analysis of SCA10 ATTCT repeat expansions among ataxia patients.** *P. Fang<sup>1</sup>, W. Jin<sup>1</sup>, T. Matsuura<sup>2</sup>, T. Ashizawa<sup>2</sup>, B.B. Roa<sup>1</sup>.* 1) Baylor DNA Diagnostic Laboratory, Molecular and Human Genetics; 2) Department of Neurology, Baylor College of Medicine, Houston, TX.

Autosomal dominant cerebellar ataxias are a heterogeneous group of neurodegenerative disorders with overlapping clinical findings. The specific diagnosis for spinocerebellar ataxia often relies on DNA analysis of various SCA genes containing unstable repeat expansion mutations (CAG repeats for SCA1, 2, 3, 6, 7 and DRPLA, CTG repeats for SCA8). SCA type 10 is associated with very large expansions of a pentanucleotide repeat (ATTCT) in the novel SCA10 gene at 22q13-qter. A normal range of 10-22 repeats was reported for this highly polymorphic repeat (heterozygosity ~80%). SCA10 patients were previously reported to have expansions ranging from ~800 to ~4500 ATTCT repeats. To date, SCA10 expansion mutations have been documented exclusively in affected individuals of Mexican ancestry. To determine the frequency of the SCA10 ATTCT expansion mutation in a cohort of ataxia patients, we analyzed a total of 314 DNA samples from symptomatic individuals who were previously referred for clinical testing to the Baylor DNA Diagnostic Laboratory. Patients who had tested negative for SCA1, SCA2, SCA3, SCA6 or SCA7 expansion mutations were subsequently analyzed for SCA10. All DNA samples were initially tested by PCR analysis across the ATTCT repeat region. A total of 250 ataxia patients were identified to have two normal alleles, and 64 patients showed one normal allele (consistent with normal heterozygosity estimates, H=80%). These 64 patient samples were further tested by Southern analysis to assess the presence of SCA10 ATTCT expansion mutations. Southern analysis identified one ataxia patient who was positive for an SCA10 expansion of ~420 ATTCT repeats. This allele represents the smallest SCA10 expansion identified in an ataxia patient to date, and appears to define the lower limit of the SCA10 mutation range. The observed SCA10 expansion frequency of ~0.3% (1/314 patients) suggests a limited contribution of SCA10 to the overall ataxia patient caseload in the United States. Furthermore, these data underscore the need for further studies on genotype-phenotype correlations for SCA10.

**Analysis Molecular In Patients With Friedreichs Ataxia In Colombian Individuals.** *M. Gomez<sup>1</sup>, C. Duran<sup>1</sup>, O. Pedraza<sup>3</sup>, E. Espinosa<sup>4</sup>, P. Vega<sup>5</sup>, M. Penaranda<sup>4</sup>, J. Prieto<sup>1,2</sup>.* 1) Instituto de Genetica Humana, Universidad Javeriana, Bogota, Bogota, Colombia; 2) Hospital La Victoria, Dpto de Gentica, Bogot, Colombia; 3) Hospital San Ignacio,Dpto Neurologia, Bogota, Colombia; 4) Hospital Militar Central, Bogota, Colombia; 5) Hospital la Samaritana, Bogota, Colombia.

Friedreichs Ataxia (FRDA), an autosomal recessive neurodegenerative disorder, is the most common hereditary ataxia. FRDA is associated with the expansion of a GAA trinucleotide repeat in the first intron of the X25 gene. We studied 24 patients with FRDA according to the essential diagnostic criteria. 17 Patients had typical FRDA and 7 had FARR. The patients showed a range in the age of onset from 3-22 years. All patients had gait, limb ataxia, dysarthria and Babinsky sign, sensory axonal polyneuropathy (62.5%), Absent tendon reflexes in the legs (75%), Nystagmus (50%), Pes cavus (37.5%) and scoliosis (50%). The GAA repeats range in the normal colombian individuals was 0-26 repeats. Expand GAA repeats were found on both alleles of the frataxin gene in 20 patients. One patient was heterozygous for the expansion mutation and was known or expected carrier of a point mutation on the other allele. In the 20 patients who were homozygous for the GAA expansion, the repeats numbers ranged from 206 to 951. The mean numbers of GAA repeats on the smaller and the larger alleles were 650 and 709 respectively. We determined the correlations between the size of the smaller expansions and both age at onset and several of disease. An inverse relation was found between the size the smaller GAA expansion and age at onset and direct relation was found between the size the smaller GAA expansion and several of disease. We investigated the single nucleotide polymorphism FAD1 in patients, indicate a strong association of the rare allele B to the FRDA chromosomes. We consider a single founder effect mutation associated with FAD1B. We demonstrated that the spectrum of FRDA is broader in the Colombian population. Direct molecular diagnosis through determination of the size of the GAA expansion should become an essential tool in clinical practice and genetic counseling for patients with recessive or sporadic cerebellar ataxias.

**ATTCT repeat instability in spinocerebellar ataxia type 10 (SCA10).** *T. Matsuura*<sup>1</sup>, *P. Fang*<sup>2</sup>, *A. Rasmussen*<sup>3</sup>, *R.P. Grewal*<sup>4</sup>, *M.E. Alonso*<sup>3</sup>, *B.B. Roa*<sup>2</sup>, *T. Ashizawa*<sup>1</sup>. 1) Neurology, Baylor College of Medicine, Houston, TX; 2) Baylor DNA Diagnostic Laboratory, Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Department of Neurogenetics, Instituto Nacional de Neurologia y Neurocirugia, Mexico City, Mexico; 4) New Jersey Neuroscience Institute, JFK Medical Center, Edison, NJ.

Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant progressive disorder characterized by ataxia, seizures and anticipation. The mutation of SCA10 is an unstable and massive expansion of an ATTCT pentanucleotide repeat (800~4500 repeats) in intron 9 of the novel gene *SCA10*. Previous studies have shown that intergenerational changes of the expanded alleles were highly variable. To understand the mechanisms underlying the variable transmission, we investigated ATTCT repeat expansions pattern through parent-of-origin transmission, somatic and germline instability, and size variability with aging. Our study showed a wide range of both allele expansion and contraction when the mutation is transmitted through the male germ line. The mean value of the repeat variation was +280 (+/-1540) repeats observed in 15 cases of paternal transmission, in contrast to an average increase of +60 (+/-120) repeats seen in 8 cases of maternal transmission ( $p<0.02$ ). Our studies also found evidence of somatic instability. In 3 out of 4 patients studied, allele contractions were observed in lymphoblastoid cells (-140 to -780 repeats) but not in blood or buccal samples. Consistent with the repeat instability observed with male transmission, analysis of sperm in two male patients showed variable expansions and contractions of the ATTCT expanded repeats (+520 to -1840 repeats). Lastly, analysis of SCA10 repeat expansion stability over time showed that the size of the expanded allele in one patient's blood lymphocytes increased by 400 repeats over a two year period. We conclude that: (1) paternal transmission is highly unstable in contrast to maternal transmission, (2) there is both somatic and germline mosaicism of the ATTCT repeat, and (3) the SCA10 repeat expansions may undergo variation with aging.



**Cloning and characterization of a CAG/CTG trinucleotide repeat expansion associated with a disorder similar to Huntington's disease.** *S.E. Holmes<sup>1</sup>, E. O'Hearn<sup>1</sup>, A. Rosenblatt<sup>1</sup>, C. Callahan<sup>1</sup>, H. Hwang<sup>1</sup>, R. Ingersoll-Ashworth<sup>1</sup>, G. Stevanin<sup>2</sup>, A. Brice<sup>2</sup>, A. Fleischer<sup>1</sup>, N. Potter<sup>3</sup>, C.A. Ross<sup>1</sup>, R.L. Margolis<sup>1</sup>.* 1) Johns Hopkins University, Baltimore, MD, USA; 2) Hôpital de la Salpêtrière, Paris, France; 3) University of Tennessee, Knoxville, TN, USA.

We recently reported a pedigree in which an autosomal dominant disorder similar to Huntington's disease (termed Huntington's disease-like 2, HDL2) segregates with an uncharacterized CAG/CTG expansion. Affected individuals tested negative for all known repeat expansions. We have now cloned the HDL2 repeat and flanking regions, and developed a PCR assay of repeat length. Unexpanded alleles in patients and controls contain repeats that range in length from 7 to 26 triplets. All affected individuals in the pedigree also have an expanded repeat of 51-57 triplets. We have now found HDL2 expansions of 44-50 triplets in three additional unrelated probands with unexplained HD-like phenotypes. The repeat, in the CTG orientation, occurs in a variably spliced exon of a brain-specific gene encoding a protein that is involved in calcium flux. Despite our previous finding of intranuclear inclusions stained by an antibody known to detect long stretches of polyglutamine (1C2) in the one available HDL2 brain, we have thus far not found evidence of a gene with the repeat in the CAG orientation. The reasons for this discrepancy remain unclear; an exon containing the repeat in the CAG orientation may have escaped detection, or 1C2 may stain material in the intranuclear inclusions other than long stretches of polyglutamine. Overall, our results suggest that the HDL2 expansion may cause selective death of striatal neurons, perhaps by a pathogenic process distinct from that of HD and other neurodegenerative disorders.

Program Nr: 2772

**Decrease of proteasome activity is age dependent and promotes nuclear accumulation of polyglutamine proteins.**

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Expanded polyglutamine tracts cause proteins to accumulate and aggregate in the nucleus, a pathological feature common to eight glutamine repeat disorders that show late-onset neurodegeneration. In Huntingtons disease (HD) mice, the disease protein huntingtin accumulates and aggregates in the nuclei of striatal neurons as the animals age. This age-dependent nuclear phenotype precedes neurodegeneration, suggesting that an age-related alteration in cellular function contributes to the pathogenesis of HD. Since the proteasome degrades misfolded and aggregated proteins, we studied the relationship between proteasome activity and the nuclear accumulation of mutant huntingtin. Striatal neurons of aged HD mice had lower proteasome activity and more intranuclear huntingtin accumulation. Similarly, primary striatal neurons cultured for a longer time had less proteasome activity and, when transfected with mutant huntingtin, more intranuclear mutant huntingtin. Moreover, inhibition of proteasome activity increases the nuclear accumulation of mutant huntingtin in stably transfected HEK 293 cells, leading to decreased cell viability specific to the mutant huntingtin. Proteasome inhibition also increases the nuclear accumulation of the DRPLA protein, another polyglutamine disease protein. We propose that an age-dependent decrease in proteasome function may contribute to the late-onset nuclear accumulation of polyglutamine proteins.

**SCA8 Expansion in the Spanish Population Including one Homozygous Patient.** *M. Mila<sup>1</sup>, B. Tazon<sup>1</sup>, E. Muñoz<sup>2</sup>, D. Jimenez<sup>1</sup>, C. Badenas<sup>1</sup>.* 1) Serv Genetica, Hosp Clinico, Barcelona, Catalonia, Spain; 2) Serv Neurologia, Hosp Clinico, Barcelona, Catalonia, Spain.

Controversial data have been reported about SCA8 since its description in 1999. The most accepted hypothesis is that CTA/CTG combined repeat expansion in the SCA8 locus causes SCA8. It is inherited as a dominant trait with reduced penetrance. The aim of the present study was to investigate the mutation incidence in the Spanish population and its possible pathogenic role. 512 chromosomes from the Spanish population, belonging to controls (298) and ataxic patients (214). DNA was extracted from blood samples by standard methods. Amplification of the CTA/CTG 3untranslated region was achieved by PCR using primers SCA8-F4 and SCA8-R3 and conditions previously described. Neurological reevaluation was done in individuals carrying expanded alleles. We have detected 5 expanded unrelated alleles corresponding to 3 affected patients and 1 healthy individual. SCA8 represents a 4.4% of total dominant spinocerebellar ataxias studied in the Spanish population. One of these patients was homozygous for the expansion. He is a 25-year-old man with clinical picture of progressive ataxia and dysarthria that began at age 12. On neurological examination he showed ataxia, slight dysarthria and nystagmus to the extreme lateral gaze. Cranial MRI showed global atrophy of cerebellum but the brainstem was spared. Family history showed the presence of ataxia in his grandfather and father. His mother is healthy at age 52 and molecular study of SCA8, reveals one allele that could be considered as premutated. She has no ataxia antecedents in her family. The healthy individual carrying an expanded allele corresponds to a control. Our results are in agreement with CTA/CTG expansion in the SCA8 locus as responsible for the SCA8 ataxia showing reduced penetrance. Homozygous status advancing age at onset supports this idea.

**Friedreich ataxia and frataxin levels.** *C. Miranda*<sup>1,2</sup>, *M. Santos*<sup>1,2</sup>, *K. Ohshima*<sup>1</sup>, *J. Smith*<sup>3</sup>, *M. Koenig*<sup>4</sup>, *J. Kaplan*<sup>3</sup>, *M. Pandolfo*<sup>1</sup>. 1) CHUM-Notre Dame Hosp, Montreal, Quebec, Canada; 2) UnIGENE, IBMC, Porto, Portugal; 3) Dept of Pathology, Univ. Utah, Salt Lake City, UT; 4) IGBMC/INSERM, Illkirch, France.

Friedreich ataxia (FRDA) is the most common autosomal recessive ataxia. The majority of patients are homozygous for a (GAA)<sub>n</sub> expansion in intron 1 of the frataxin gene. The size of this expansion ranges from 100 to more than 1000 triplets, resulting in decreased levels of frataxin expression (4 to 29% of non affected individuals). Frataxin is a highly conserved mitochondrial protein that is thought to prevent toxic iron accumulation in mitochondria. In the frataxin knockout mouse absence of frataxin expression causes embryonic lethality a few days after implantation (E6), showing that at least a residual amount of frataxin has to be present for the embryo to be able to survive the embryonic period. Heterozygous knockout mice show a normal embryonic development, and do not develop any symptoms of the disease. With the aim to generate a *frda* mouse model that would express levels of frataxin close to the range observed in FRDA patients, we have generated a *frda* knock-in mouse by insertion of a 230GAA repeat expansion in intron 1 in the murine *frda* gene. The repeat is stably transmitted and does not show somatic instability. Homozygous *frda* knock-in mice show a mild reduction of expression of frataxin when compared with wild type mice. To further decrease the level of frataxin expression, we crossed heterozygous knock-in mice with heterozygous knockout mice to give double heterozygotes that show levels of expression of frataxin close to 25% of levels found in wild type mice. These animals are phenotypically normal at 12 months of age, in particular do not show reduced motor coordination at the rotarod test, suggesting that this level of frataxin is sufficient for normal mitochondrial iron homeostasis. Pathological and biochemical studies are in progress. We are also currently studying whether these mice respond normally to iron loading.

**A Mouse Model for Spinal and Bulbar Muscular Atrophy.** *C.J. O'Brien, E. Chevalier-Larson, S. Jenkins, D.E. Merry.* Biochemistry and Molecular Pha, Thomas Jefferson University, Philadelphia, PA.

Spinal and Bulbar Muscular Atrophy is an adult onset, slowly progressive motor neuropathy caused by an expansion of a polyglutamine tract within the androgen receptor (AR) protein. We have created an animal model of SBMA, using transgenic mice that bear the full-length mutant or normal AR cDNA driven by the prion protein (PrP) promoter. Preliminary characterization of transgenic mice revealed signs of neurologic disease. These signs include reduced activity and limb clasping, which was notably progressive, beginning with isolated hindlimb clasping at two months and progressing to forelimb clasping, then to a full body clasp by 8 months. This behavior was not observed in non-transgenic littermates, nor in any normal repeat transgenic mice. Also, clasping was seen in male transgenic mice as young as two months, but only observed in the mildest form in female mice of the same line beginning at 8 months of age. The PrP-AR112-27 transgenic line revealed clasping at 12 months of age, and an increased rate of early death. Another founder had progressive hindlimb gait abnormalities at 3 months of age. F1 mice from this founder are under study. Immunohistochemical analysis of brain and spinal cord from six month old transgenic PrP-AR112-34 animals revealed nuclear staining in most neurons of the brain and spinal cord. Also, intranuclear inclusions were identified in neurons of transgenic cerebral cortex and spinal cord with the N-terminal antibody AR(N-20) and with antibody AR318. Aggregates were not detected with antibody AR (C-19), indicating that inclusions detected in these transgenic mice resemble those of Kennedy's disease nervous tissue in the absence of C-terminal epitopes. The presence of selected epitopes in neuronal inclusions provides a system with which to investigate the role of AR proteolysis in the onset and progression of Kennedy's disease. The male-specific neurologic phenotypes observed in at least one line of transgenic mice likely result from the same pathogenic mechanism as in Kennedys disease and provides us with an appropriate model to test the effects of various AR ligands on phenotype onset and progression.

**A survey of *TWIST* for mutations in craniosynostosis reveals a variable length polyglycine tract in asymptomatic individuals.** A.O.M. Wilkie<sup>1,4</sup>, N. Elanko<sup>1</sup>, J.S. Sibbring<sup>2</sup>, K.A. Metcalfe<sup>2</sup>, J. Clayton-Smith<sup>2</sup>, I.K. Temple<sup>3</sup>, S.A. Wall<sup>4</sup>, D. Donnai<sup>2</sup>. 1) Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, UK; 2) Regional Genetic Service, St Mary's Hospital, Manchester, UK; 3) Wessex Clinical Genetics Service, The Princess Anne Hospital, Southampton, UK; 4) Oxford Craniofacial Unit, Radcliffe Infirmary, Oxford, UK.

The human *TWIST* gene encodes a 202 amino acid transcription factor characterized by a highly conserved basic-helix-loop-helix motif in the C-terminal half, and a less conserved N-terminal half that has binding activity towards the histone acetyltransferase p300. Between these domains is a repeat region of unknown function that encodes the glycine-rich sequence (Gly)<sub>5</sub>Ala(Gly)<sub>5</sub>. Heterozygous mutations of *TWIST* were previously described in the Saethre-Chotzen craniosynostosis syndrome.

During a search for *TWIST* mutations in patients with craniosynostosis, we identified, in addition to ten novel and one previously described *bona fide* intragenic mutations and one complete gene deletion, 13 individuals from 7 independently ascertained families with rearrangements of the glycine-rich region. These involved either deletion of 18 nucleotides (259\_276del18) or insertion of 3 (259\_260ins3), 15 (274\_275ins15) or 21 (276\_277ins21) nucleotides. These rearrangements converted the G<sub>5</sub>AG<sub>5</sub> sequence to G<sub>5</sub>, G<sub>6</sub>AG<sub>5</sub>, G<sub>5</sub>AG<sub>4</sub>AG<sub>5</sub>, and G<sub>5</sub>AG<sub>6</sub>AG<sub>5</sub>, respectively. None was consistently associated with clinical disease and we conclude that they are at most weakly pathogenic.

Less is known about the pathophysiology of variation in polyglycine repeats, compared to polyglutamine or polyalanine repeats. In the case of *TWIST* protein, the glycine stretch may serve as a flexible linker between the functional domains, and as such may be subject to reduced evolutionary constraint.

**A Sensitive Balance of Ataxin-2 Activity is Required for Proper Actin-Cytoskeletal Structure and Tissue Viability in *Drosophila*.** *T.F. Satterfield, L.J. Pallanck.* .

Spinocerebellar Ataxia Type 2 (SCA2) is a member of a class of neurodegenerative diseases caused by the expansion of an endogenous glutamine repeat in the coding sequence of the corresponding gene. The normal cellular function of the *SCA2* gene product, ataxin-2, and the mechanism by which polyglutamine expansion of any of the genes in this class of disorders causes neurodegeneration remain unknown. To assess the possible contribution of altered ataxin-2 function to *SCA2* pathology we have used a genetic approach to study a *Drosophila* ortholog of the *SCA2* gene (termed *datx2*). Mutations that reduce *datx2* activity, or transgenic overexpression of *datx2* results primarily in lethality with survivors typically displaying locomotor defects and tissue degeneration. Subcellular examination of tissues affected by altered *datx2* gene dosage reveals defects in actin filament organization and the appearance of polymerized actin aggregates. Together, these results demonstrate that *datx2* participates in actin filament formation, and that a sensitive balance of *datx2* activity is required for viability. These findings, when coupled with the observation that loss of cytoskeletal-dependent neuronal structure defines an early event in *SCA2* pathogenesis, support a mechanism of *SCA2* pathology involving actin cytoskeletal defects resulting from alteration of ataxin-2 activity.

**Mutations in *CRBI* are a major risk factor for the development of Coats-like exudative vasculopathy in retinitis pigmentosa, and are the cause of 13% of Leber congenital amaurosis.** A.I. den Hollander<sup>1</sup>, J.R. Heckenlively<sup>2</sup>, L.I. van den Born<sup>3</sup>, Y.J.M. de Kok<sup>1</sup>, S.D. van der Velde-Visser<sup>1</sup>, U. Kellner<sup>4</sup>, B. Jurklies<sup>5</sup>, M.J. van Schooneveld<sup>6</sup>, K. Rohrschneider<sup>7</sup>, B. Wissinger<sup>8</sup>, J.R.M. Cruysberg<sup>9</sup>, A.F. Deutman<sup>9</sup>, H.G. Brunner<sup>1</sup>, E. Apfelstedt-Sylla<sup>8</sup>, C.B. Hoyng<sup>9</sup>, F.P.M. Cremers<sup>1</sup>. 1) Dept. of Human Genetics, University Medical Centre Nijmegen, Nijmegen, Netherlands; 2) Dept. of Ophthalmology, University of California, Los Angeles, CA; 3) The Rotterdam Eye Hospital, Rotterdam, Netherlands; 4) Dept. of Ophthalmology, University Clinic Benjamin Franklin, Berlin, Germany; 5) Universitäts-Augenklinik, Essen, Germany; 6) Dept. of Ophthalmology, University Medical Centre Utrecht, Utrecht, Netherlands; 7) Universitäts-Augenklinik, Heidelberg, Germany; 8) Universitäts-Augenklinik, Tübingen, Germany; 9) Dept. of Ophthalmology, University Medical Centre Nijmegen, Nijmegen, Netherlands.

Previously we described mutations in the crumbs homologue 1 *CRBI* gene in a severe form of retinitis pigmentosa (RP12), which is characterized by a preserved para-arteriolar retinal pigment epithelium. To determine whether *CRBI* mutations are a common cause of retinal dystrophies, a mutation screen was performed in 97 patients with RP and 52 patients with Leber congenital amaurosis (LCA).

In the RP group, mutations were detected in a patient who had developed a Coats-like exudative vasculopathy, a relatively rare complication of RP that may progress to partial or total retinal detachment. Screening of 8 more patients who had developed this complication revealed mutations in 4 of them. Given that 4 out of 5 patients had developed the complication in one eye and that not all siblings with RP have the complication, *CRBI* mutations should be considered an important risk factor for the Coats-like reaction, although its development may require additional genetic or environmental factors.

Mutations were also detected in 7 (13%) of 52 LCA patients. In three patients we identified null mutations on both *CRBI* alleles, which suggests that LCA is the most severe phenotype that can be associated with mutations in *CRBI*.



**CDH23 mutational and clinical analyses: a continuum of genotype/phenotype relationships in a cohort of 94**

**Usher Id and DFNB12 patients.** *L.M. Astuto<sup>1</sup>, R.R. Fields<sup>1</sup>, J.W. Askew<sup>1</sup>, J.M Bork<sup>2</sup>, R.J. Morell<sup>2</sup>, H. Kremer<sup>3</sup>, P. van Hauwe<sup>3</sup>, W.J. Kimberling<sup>1</sup>.* 1) Gene Marker Laboratory, Boystown National Research Hospital, Omaha, NE USA; 2) Laboratory of Molecular Genetics, National Institute on Deafness and Other Communication Disorders, NIH, Rockville, MD, USA; 3) Department of Otorhinolaryngology, University Hospital, Nijmegen, the Netherlands.

Usher syndrome, the most common type of hereditary deaf-blindness, represents a heterogeneous group of autosomal recessive disorders characterized by sensorineural hearing impairment, progressive retinal degeneration and in some cases vestibular areflexia. Usher type Id (OMIM 601067), the fourth of six Usher I genetic localizations, was first mapped to 10q21-22 (Wayne et al. 1996), a region that overlaps DFNB12, a non-syndromic deafness localization (Chaib et al. 1996). Mutations in CDH23, a cell adhesion protein with multiple cadherin-like domains, are responsible for both syndromic (Usher Id) and non-syndromic (DFNB12) deafness (Bork et al. 2001). Specific CDH23 mutational defects were identified distinguishing these two phenotypes as only milder missense mutations were detected in DFNB12 families and nonsense and frameshift mutations were identified in Usher Id families. In this study, all 69 exons of CDH23 were amplified in 94 genetically independent and ethnically diverse Usher I and non-syndromic deafness probands. Mutation detection was assessed by heteroduplex analysis and direct sequencing. Our results have consistently verified and expanded the mutational specific observations of mild and severe mutations in DFNB12 and Usher Id families as previously reported. In addition, a thorough and extensive clinical evaluation of the CDH23 mutant families has revealed typical Usher I cases, as well as atypical and complex Usher I phenotypes manifesting variable hearing and vestibular involvement, a late to mild onset of retinitis pigmentosa and subnormal ERG retinal findings not entirely consistent with a non-syndromic deafness phenotype. These findings warrant a critical clinical evaluation of the phenotypic variation and type of mutation involved for accurate diagnosis of non-syndromic or syndromic hearing loss.

**Duplication as well as haploinsufficiency of the forkhead/winged-helix transcription factor FOXC1 cause human anterior segment dysgenesis.** *F. Mirzayans*<sup>1</sup>, *R. Saleem*<sup>1</sup>, *D.B. Gould*<sup>1</sup>, *J. Marshall*<sup>1</sup>, *O. Lehmann*<sup>2</sup>, *T. Jordan*<sup>2,3</sup>, *V.*

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The human FOXC1 gene, a member of forkhead winged-helix family of transcription factors mapping to chromosome 6p25 has been previously implicated in the development of anterior segment of the eye. To date 16 different mutations of the FOXC1 have been detected in individuals with anterior segment dysgenesis and glaucoma (Mears et al., 98; Nishimura et al., 2001), including two frameshift and two nonsense mutations consistent with a haploinsufficiency model underlying FOXC1-associated ocular defects. However, mutational analysis of FOXC1 has not revealed any disease-associated alterations in two of our large families originally linked to 6p25. Analyses involving newly derived polymorphic markers have limited the critical region to 300 kb of genomic sequence. In addition to FOXC1, two other forkhead genes FOXF2 and FOXQ1 map within this critical interval at 6p25. Direct sequencing analyses detected polymorphisms in both the FOXF2 and FOXQ1 genes, but failed to detect any disease-causing alterations in our families. In the light of recent evidence pointing to duplications of the 6p25 region also associated with ocular defects (Lehmann et al., 2000; Nishimura et al., 2001), we reexamined our linkage data and noticed a subtle difference in intensity in the alleles segregating with the disease in these two families in markers clustering around FOXC1 and FOXF2 genes. Preliminary FISH and Southern blot analyses are consistent with the possibility of a small duplication of the region including FOXC1, in affected members of these two kindreds. These findings strongly suggest that FOXC1 normally functions within a very narrow threshold window, and that perturbations resulting in either too little or too much FOXC1 activity result in similar anterior segment defects.

**Mutations in the *Drosophila* homolog of the gene for primary congenital glaucoma cause fluid flow and neurological defects.** *L.T. Reiter*<sup>1</sup>, *M. McElroy*<sup>1</sup>, *B.B. Bejjani*<sup>2</sup>, *E. Bier*<sup>1</sup>. 1) Dept. Biology, UCSD, La Jolla, CA; 2) Baylor College of Medicine, Houston, TX.

Mutations in the human cytochrome P450 *CYP1B1* are known to result in primary congenital glaucoma (PCG). One of the characteristics of this disorder is a developmental defect resulting in an obstruction of fluid flow through the trabecular meshwork, increased intraocular pressure and optic nerve damage. *cyp18a* is the C-P450 gene in *Drosophila melanogaster* that is most similar to human *CYP1B1*. Misexpression of *cyp18a* in the wing results in a significant narrowing of the wing margin and ectopic wing vein material. Ubiquitous misexpression of *cyp18a* can cause lethality and wing malformations at several timepoints during pupal development. Mutation of the *cyp18a* gene on the X chromosome by *p*-element insertions at two different locations resulted in two distinct sets of phenotypes. Class I alleles are semi-lethal in males (1:100), have wing hinge defects, and appear to have a neurological defect which affects coordination and walking, while class II alleles are viable but exhibit fluid flow defects manifested as deflated wings and bloated abdomens filled with hemolymph. We screened a collection of 1300 *p*-element insertions for dominant suppressors of the class I *cyp18a* mutant phenotype and have thus far identified at least 16 genes which, when disrupted in the heterozygous state, can at least partially rescue the male semi-lethality. Three of these genes are known to be involved in cell adhesion: *vkg*, *Ten-m* and *dally*. We also identified a gene called *ttv* which is very similar to a tumor suppressor gene located on human chromosome 8p (the region in humans to which a suppressor of *CYP1B1* mutations is linked). We are currently investigating the role of apoptosis in the generation of the class II fluid flow defect and the semi-lethality. Preliminary results suggest that *cyp18a* is involved in the regulation of a small molecule which may direct apoptosis in the hinge and veins of the wing. These results raise the possibility that human *CYP1B1* may modify or produce a similar small molecule which is responsible for inducing normal apoptosis in the trabecular meshwork of the eye.

**A mutation in the RX homeobox gene in 2 individuals with different ophthalmological manifestations.** A.S. Schneider<sup>1</sup>, M.A. Dwyer<sup>1</sup>, V. Voronina<sup>2,5</sup>, C. O'Kernick<sup>2,5</sup>, P. Mathers<sup>2,3,4,5</sup>. 1) Dept Genetics, Albert Einstein Medical Ctr, Philadelphia, PA; 2) Dept Biochemistry, West Virginia University School of Medicine, Morgantown, WV; 3) Dept Otolaryngology, West Virginia University School of Medicine, Morgantown, WV; 4) Dept Ophthalmology, West Virginia University School of Medicine, Morgantown, WV; 5) Sensory Neuroscience Research Center, West Virginia University School of Medicine, Morgantown, WV.

The anophthalmia/microphthalmia (A/M) registry at Albert Einstein Medical Center, has been collecting data on individuals with A/M for 7 years and in the past 2 years has offered gene screening to registry participants. Testing involves mutational screening of several eye development genes. We have identified two patients from this screen who carry a mutation of the RX homeobox gene. Patient 1 is a 3 10/12 year old male with unilateral Persistent Hyperplastic Primary Vitreous (PHPV) and microphthalmia of the left eye. The right eye is completely normal. He has no other anomalies and his development is appropriate for age. Patient 2 is an 8 year old female with left anophthalmia, right microphthalmia with supranasal coloboma of iris, chorioretinal coloboma and hypoplastic optic nerve, global developmental delay and an ASD. Some autistic features have been noted.

Both individuals are heterozygous for a C-to-T mutation at position 147 of the RX homeobox gene. This mutation introduces a premature termination codon into helix 1 of the homeodomain, presumably preventing the protein binding activity of the native protein. Sequence analysis of the RX coding region in both affected individuals failed to identify a second mutation, though genetic evidence from a third family suggests that a single copy of the Q147X mutation is not sufficient by itself to cause abnormal eye development. DNA testing of the parents for both patients suggests that these mutations arose spontaneously. We are continuing efforts to identify the site of a potential second mutation in the RX gene. Together with data from a mouse Rx-gene deletion model, these data implicate the RX gene as crucial for the proper formation of the mammalian eye.

**Familial vestibulocerebellar disorder maps to chromosome 13q31-q33: a new nystagmus locus.** *N.K. Ragge<sup>1</sup>, C. Hartley<sup>2</sup>, A.M. Dearlove<sup>2</sup>, J. Walker<sup>3</sup>, I. Russell-Eggitt<sup>3</sup>, C.M. Harris<sup>4</sup>.* 1) St Thomas' Hospital, London, England; 2) Medical Research Council Human Genome Mapping Project Resource Centre, Cambridge; 3) Dept of Ophthalmology, Great Ormond St Hospital for Children, London; 4) Institute of Neuroscience, Plymouth University.

Pathological nystagmus (involuntary oscillation of the eyes) usually occurs in three settings: 1) onset at birth or in the first few months associated with ocular disease (sensory defect nystagmus); 2) a similar early onset with no detectable underlying pathology (congenital idiopathic nystagmus); and 3) acquired (onset after 6 months) associated with a neurological disorders (neurological nystagmus). We present a unique 4-generation family with dominantly inherited nystagmus that develops in the first year of life. The family was initially published several years ago (OMIM 193003), but the pedigree has enlarged since this time. We have performed a whole genome search and localized the disorder to chromosome 13q31-q33. Haplotype construction and analysis of recombination events narrowed the region to a 13.8 cM region between markers D13S1300 and D13S158. Potential candidate genes in this region include SOX21, TYRP2 and ZIC2. Identification of the gene may hold the key to understanding pathways for early eye movement development.

**Aryl-hydrocarbon receptor interacting protein-like 1 (AIPL1): a possible function in photoreceptor-specific regulation of apoptosis.** *M.M. Sohocki<sup>1</sup>, S.P. Daiger<sup>1</sup>, D.T. Akey<sup>1</sup>, X. Zhu<sup>2</sup>, C. Craft<sup>2</sup>*. 1) Hum Genet Ctr, Univ TX Health Sci Ctr, Houston, TX; 2) USC Keck School of Med, Doheny Eye Inst, Los Angeles, CA.

*AIPL1* was the fourth gene to be associated with Leber congenital amaurosis (LCA), and the AIPL1 protein contains functional motifs commonly identified in chaperone proteins. In order to determine potential targets for this predicted activity of AIPL1, a yeast-two hybrid (Y2H) approach was used.

Screening of a bovine retinal cDNA library identified two potential Aipl1-interacting proteins, aven and Nub1, three and two times, respectively. These interactions are undergoing further confirmation using co-immunoprecipitation, immunolocalization, and GST pull-down using both the bovine and human proteins.

Both Aven and NUB1 are recently identified proteins that are likely involved in regulation of apoptosis within the retina. Aven is a cell death regulator that interferes with the ability of Apaf-1 to self-associate, suggesting that it impairs the Apaf-1-mediation of the caspases. NUB1 inhibits expression of NEDD8, a protein involved in G1/S progression of the cell cycle. Apoptosis and the cell cycle are closely tied together, and re-expression of cell cycle markers has been linked to certain types of neuronal cell death. As the photoreceptor cell is committed to permanent cessation of cell division, it is thought that forced reentry of the photoreceptor into the cell cycle after this commitment causes cell death.

If confirmed, AIPL1 will be the first photoreceptor-specific regulator of apoptosis to be identified, and will likely be an integral gene and protein in the development of treatments for inherited retinal degenerations. These treatments might involve therapies for multiple forms of inherited blindness, because in all forms of human retinal degeneration studied, photoreceptors eventually degenerate by the apoptotic pathway, and in at least some forms of inherited retinopathy, photoreceptor degeneration may be prevented or slowed by treatment with agents that interfere with apoptosis.

**Fine mapping of the Cerulean Cataract Type 1 gene locus on 17q24-q25.** *B.W. Rigatti<sup>1</sup>, F.Y. Demirci<sup>1,2</sup>, R.E. Ferrell<sup>2</sup>, M.B. Gorin<sup>1,2</sup>.* 1) Dept. of Ophthalmology, University of Pittsburgh SOM, Pittsburgh, PA; 2) Dept. of Human Genetics, University of Pittsburgh GSPH, Pittsburgh, PA.

Cerulean cataracts represent one of many forms of autosomal dominant, early onset hereditary cataracts and are characterized by the appearance of tiny blue or white opacities, predominantly in the peripheral layers of the lens. There are two loci reported for this distinctive phenotype: cerulean cataract type 1 on 17q24-q25 and cerulean cataract type 2 on 22q11.2-q12.2. Type 2 has been shown to be caused by mutation in the beta-B2-crystallin gene. The type 1 locus is flanked by microsatellite markers D17S802 and D17S836 and no causative gene has been identified. The family originally mapped to 17q24-q25 contains individuals with 6 recombinations within the flanking markers, but further localization has been hampered by the lack of informative markers. To narrow the interval, we have used GenBank and other genomic databases to generate a preliminary BAC contig. Various ESTs, STSs and SNPs have been identified within this region using both the Human Genome Project and Celera databases. Sixteen SNPs spanning our critical region have been analyzed by first testing within two distinct branches of the family that include the key recombinant individuals, screening with nucleotide sequencing, and then followed by more extensive screening of the entire family with denaturing HPLC (Transgenomic Wave). Twelve out of the sixteen SNPs were polymorphic in some of the family members. Eleven SNPs are informative and have narrowed the critical region containing this locus so that we can evaluate a more limited set of candidate genes for mutations. To date, we have screened two genes and no mutations have been identified. The screening of the remaining genes in the critical region is underway.

**Molecular Basis for Reduced Penetrance in Primary Congenital Glaucoma.** *I. Stoilov<sup>1</sup>, I. Jansson<sup>2</sup>, J.B.*

*Schenkman<sup>2</sup>, M. Sarfarazi<sup>1</sup>.* 1) Molecular Ophthalmic Genetics Laboratory, Department of Surgery; 2) Department of Pharmacology, UConn Health Center, Farmington, CT.

The severe primary congenital form of glaucoma (PCG) is familial in 10-40% of index cases. In majority of the reported families, PCG is inherited as autosomal recessive trait. However, in some cases the number of affected sibs is lower than expected thus indicating the possibility of reduced penetrance for this phenotype. Such possibility has recently been supported by the molecular analysis of PCG, the CYP1B1 gene in the Saudi Arabian population. Here we report our progress in the characterization of two CYP1B1 mutations: G61E and R469W, which have been associated with reduced penetrance of the PCG phenotype. G61E is located within the hinge region of CYP1B1 that permits flexibility between the membrane-spanning domain and the cytoplasmic portion of the molecule while R469W is located in the heme-binding region. We created these two mutations in CYP1B1 cDNA by site-directed mutagenesis and expressed them in *E. coli*. Three recombinant hemoproteins CYP1B1, G61E and R469W were then compared with respect to their stability and metabolic activity. Highest stability occurred in R469W, which only exhibited 37% decline in the amount of cytochrome P450 by 48 hrs. The CYP1B1 itself lost 50% over the same period while G61E lost 55% of the hemoprotein spectrum by 5 hrs and 70% by 24 hrs. Compared to the CYP1B1, the 4-hydroxylase activity against 17-beta-estradiol was reduced by 70% and 80% for G61E and R469W respectively. The 2-hydroxylase activity was decreased 65% for R469W but only 20% for G61E. Our data suggest that both mutant forms associated with reduced penetrance have residual metabolic activity against 17-beta-estradiol. The significance of this observation is in the fact that the expression of CYP1B1 could be upregulated several folds by chemicals such as dioxin acting via the aryl hydrocarbon receptor (AhR). Therefore, environmental exposure causing AhR driven over-expression of these mutant forms during the critical stages of development may provide sufficient CYP1B1 activity for the normal eye development. Supported by: NIH (EY-11095; ES03154) and The Glaucoma Foundation.



**Genotype-phenotype correlations in a series of 26 Rett Syndrome patients with MECP2 mutations.** A. Moncla<sup>1</sup>, A. Kpebe<sup>2</sup>, B. Chabrol<sup>3</sup>, J. Mancini<sup>3</sup>, N. Philip<sup>1</sup>, L. Villard<sup>2</sup>. 1) Dept de Génétique Médicale, Hopital d'Enfants de la Timone, Marseille, France; 2) Inserm U491, Fac de Médecine de La Timone, Marseille, France; 3) Dpt de Neuropédiatrie, Hopital d'Enfants de La Timone, Marseille, France.

Rett Syndrome (RTT, MIM 312750) is a severe neurological disorder affecting exclusively females. Its prevalence is about 1 in 15,000 live born females. Rett patients stop developing at about 1 year of age, and have a series of clinical signs indicative of a neurodevelopmental abnormality : arrest of brain development, regression of acquisitions, and behavioural troubles (stereotypic hand movement, autism). Most cases are sporadic (99.5%), although a few families have been reported. Mutations in the human methyl-CpG binding protein 2 (MECP2) gene located in Xq28 were identified in 70 to 80% of sporadic Rett syndrome case. We have screened the coding region of the MeCP2 gene in 43 female patients with clinical signs evocative of Rett syndrome. We found 26 mutations (60%) affecting different regions of the MeCP2 protein : R106G, R106W (3 cases), P127L, P152R, T158M (3 cases), R168X (4 cases), R255X (4 cases), R270X (4 cases), R306C, 883delC, 1156del43, 1157del32, 1196del71+inv(1197-1238). Since several mutations affecting the same residue were found in different patients and since a detailed clinical description was available for each, we were able to compare the location and type of the mutation with the clinical signs. The X-chromosome inactivation status was also analysed in each case to determine if favourable skewing could lead to a milder phenotype.

**A Complete Gene Catalogue of Human Xp11.4 harboring Disease Loci for Diabetes Mellitus Type I, Mental Retardation and Retinal Disturbances.** *J. Ramser*<sup>1</sup>, *G. Wen*<sup>2</sup>, *Y. Demirci*<sup>3</sup>, *I. Martinez-Garay*<sup>4</sup>, *S. Engert*<sup>1</sup>, *C. Pusch*<sup>5</sup>, *K. Badenhoop*<sup>6</sup>, *M. Gorin*<sup>3</sup>, *M. Platzer*<sup>2</sup>, *A. Meindl*<sup>1</sup>. 1) Dept. of Medical Genetics, Ludwig-Maximilians-University, Munich, Germany; 2) Dept. of Genome Analysis, Institute for Molecular Biotechnology, Jena, Germany; 3) Dept. of Ophthalmology and Human Genetics, Pittsburgh, USA; 4) Dept. of Genetics, University of Valencia, Valencia, Spain; 5) Molekulargenetisches Labor, Universitaets-Augenklinik, Tuebingen, Germany; 6) Dept. of Medicine I, University of Frankfurt, Frankfurt, Germany.

Different disease entities like two syndromic forms of mental retardation, diabetes mellitus type I and two eye diseases have been mapped to the short arm of the X-chromosome including the Xp11.4 region. In order to isolate genes, involved in these conditions, we have constructed a complete BAC/PAC-contig for the Xp11.4 region between markers DXS8025 and DXS228 encompassing about 3.0 Mb. For the proximal part of this region, between DXS993 and DXS228, a complete transcription map was established. Six distinct genes were found in this 1.5 Mb large interval, four of them were screened for mutations in patients with the complete form of congenital stationary night blindness (CSNB1). A novel gene termed NYX was found to be mutated in CSNB1 families (Pusch et al., Nature Genetics 2000). NYX is widely and low expressed and codes for a 481 amino acid protein. In order to identify candidate genes for X-linked cone dystrophy (COD1) we are now analysing the 1.5 Mb large distal part of the contig (DXS8025-DXS993). Sequence analysis using different software packages, which combine gene prediction programs, the masking of repeats and homology searches against different DNA and protein databases has resulted in the identification of three genes so far. They are currently screened for mutations in COD1 families. Genes isolated from this region will also, together with genes from the adjacent fully characterized Xp21.1 region, be evaluated for variants in patients suffering from diabetes mellitus type I. Finally, all genes from the Xp11.4 region and from the proximally located regions Xp11.3 and Xp11.23, can be screened for mutations in Prieto- and Renpenning syndrome, respectively.

**Prenatal Diagnosis of Beta-thalassemia in Iranian population, results of ten years study.** *F. Sahebjam<sup>1</sup>, R. Karimi-Nejad<sup>1</sup>, S. Teimourian<sup>1</sup>, S. Sahebjam<sup>1</sup>, H. Najmabadi<sup>2</sup>.* 1) Karimi-Nejad Pathology and Genetic Center, Tehran, Tehran, Iran; 2) Genetics Research Center, University of Welfare Sciences, Tehran, Tehran, Iran.

Beta-thalassemia is the most common genetic disorder in Iran. It is estimated that at least 25,000 affected patients and 2 million carriers live in Iran. Therefore prenatal diagnosis is at present a primary goal for prevention. As a first center, 10 years ago we started mutation screening and prenatal diagnosis of beta -thalassemia in Iranian families. During 10-year period we have made prenatal diagnosis for 414 cases ( 211 amnion samples and 204 CVS samples). We used a 24 primers-based panel for diagnosing the mutations of the parents and samples. Using this panel along with RFLP we were able to provide a reliable prenatal diagnosis for over 95% of pregnancies. Out of these 414 cases, 76 (18.35%) were Normal, 188 (45.41%) cases were heterozygotes for a single beta-thalassemia mutation, and 116 (28.1%) cases were either homozygotes or compound heterozygotes. In 30 samples (7.4%) we could not detect the mutations. Our data showed a very close Mendelian distribution as expected for a Mendelian type mode of inheritance. The frequency of detected mutations also will be discussed.

**Penatal Diagnosis Of X-Linked Ichthyosis By Very Low Levels Of Maternal Serum Unconjugated**

**Oestriol(MSuE3)As Measured By The Triple Test.** *G. Bach<sup>1</sup>, S. Feinstein<sup>1</sup>, R. Bargal<sup>1</sup>, V. Suri<sup>1</sup>, I. Yonah<sup>2</sup>, O.*

*Bonne<sup>2</sup>, M. Zeigler<sup>1</sup>, Z. Ben-Neriah<sup>1</sup>.* 1) Dept Human Genetics, Hadassah Medical Organization, Jerusalem, Israel; 2) Dept Psychiatry, Hadassah Medical Organization, Jerusalem.

Since the advent of the triple test which includes the determination of unconjugated oestriol found in maternal serum (MSuE3) as a parameter for the identification of high risk pregnancies for Down Syndrome, there has been an increase in the number of detected fetuses affected with the dermatological disorder X-linked Ichthyosis (XLI). XLI is associated with very low levels of MSuE3. XLI is caused by a deficiency of the enzyme steroid sulfatase (STS). Since 85-90% of XLI males have a complete deletion of the STS gene and in ~ 5% of males the deletion of the STS gene is seen as part of a contiguous gene syndrome (CGS) giving rise to a complex phenotype, a problem arises in genetic counselling in families without prior history of the disease. To improve genetic counselling in these cases we have performed molecular studies on 21 unrelated XLI males and 20 XLI male fetuses found by very low MSuE3, 8 of whom were from families with prior history of XLI. The importance of the molecular test results and prenatal genetic counselling in these cases will be discussed. Key words: X- Linked Ichthyosis (XLI), Maternal serum unconjugated oestriol (MSuE3), Steroid sulfatase (STS), Contiguous gene syndrome (CGS).

**Clinical significance of prenatally detected Dandy-Walker malformation and variant.** *R.L. Olson<sup>1</sup>, L.H. Seaver<sup>1</sup>, R.C. Rogers<sup>1</sup>, P.C. Greig<sup>2</sup>, E.H. Dellinger<sup>2</sup>, S.J. Chapman<sup>2</sup>, R.E. Stevenson<sup>1</sup>.* 1) Greenwood Genetic Center, Greenwood, SC; 2) Greenville Hospital System, Greenville, SC.

Dandy-Walker malformation (DWM) is described as a posterior fossa cyst, cerebellar vermis defect, hydrocephalus of variable degree, and elevation of the torcula and tentorium. Dandy-Walker variant (DWV) is a less severe malformation described as isolated atresia of the cerebellar foramina, enlarged cisterna magna, or cysts that do not communicate with the fourth ventricle.

The prognosis and clinical significance of prenatally diagnosed DWM and DWV are controversial. Prognosis is heavily dependent on associated malformations and karyotype, but is not uniformly fatal [Kolble et al. 2000, Ecker et al. 2000]. We report 20 cases of prenatally diagnosed DWM (n=16) and DWV (n=4) from 1994 to 2000. For each case the prenatal ultrasound was compared with the available postnatal studies, autopsy findings, or clinical examinations. A review of the findings shows 12/20 patients are living today, but 5 patients have diagnosed MCA syndromes. The 8 patients who died had serious anomalies in addition to DWM. 4/17 who had chromosome analyses had cytogenetic abnormalities. Non-CNS anomalies were found in 11/20 patients. 3/20 had different or additional CNS defects identified. When comparing the prenatal and postnatal diagnosis, 5/13 patients were found to have normal or normal variant postnatal scans.

The findings in these patients suggest DWM is difficult to accurately diagnose prenatally. When diagnosed correctly there is a significant morbidity associated with DWM. If DWM is associated with non-CNS findings there is increased morbidity and mortality. Even apparent isolated DWM may prove to have additional defects. Since 5/13 patients had normal postnatal scans, caution should be used in prenatal counseling. Amniocentesis should be offered in all cases of prenatally diagnosed DWM. A careful postnatal genetic evaluation should be performed.

**No evidence for involvement of PPARG in neural tube defect families.** *D.G. Siegel<sup>1</sup>, E.C. Melvin<sup>1</sup>, H. Cukier<sup>1</sup>, T.M. George<sup>1</sup>, J.F. Mackey<sup>1</sup>, G. Worley<sup>1</sup>, J.S. Nye<sup>2</sup>, M.C. Speer<sup>1</sup> and NTD Collaborative Group.* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Northwestern University Medical Center, Chicago, IL.

An increase in the frequency of twins has been suggested in probands with neural tube defects (NTDs) and in close relatives of NTD probands. Reports as to whether the increase involves monozygotic or dizygotic (DZ) twinning are inconsistent. Recently, Busjahn et al. (Nat Genet 26: 398) reported that the gene encoding peroxisome activated receptor (PPARG) may be involved in the etiology of DZ twins. We hypothesized that if there is a similar mechanism influencing DZ twinning and NTDs, we would see differences in allele frequencies in NTD families when compared to controls. We looked at allele frequencies for PPARG in NTD cases, mothers of NTD cases, and fathers of NTD cases in 145 American Caucasian NTD families in which the proband had lumbosacral myelomeningocele. Each group was in Hardy-Weinberg equilibrium. There were no differences in allele frequencies between any of these groups and a series of unrelated controls, nor was there evidence for linkage disequilibrium within these families following testing via the pedigree disequilibrium test. However, when the parental allele frequencies were compared to those reported by Busjahn et al., we found a significant difference ( $p = 0.01$ ) between these groups. In our series of NTD families (N=750), we find that the rate of twinning among NTD probands and close relatives of NTD probands (e.g., siblings, parents, aunts/uncles) is not significantly higher than the general population frequency. These data suggest that the mechanism involved in DZ twinning associated with PPARG is different from the causation of lumbosacral NTDs in these American Caucasian families.

**Cardiac malformations in the first trimester fetuses with increased nuchal translucency.** *I.V. Novikova, G.L. Tsukerman, S.A. Shreder, O.V. Pribushenya, L.A. Savenko, N.A. Venchikova, L.M. Lishtvan, G.A. Krapiva, S.I. Kovalev, I.A. Kirillova.* Institute for Hereditary Diseases, MINSK, Belarus.

Pathological examination of the heart and great arteries was performed in 65 fetuses with increased nuchal translucency >3 mm. after surgical termination of pregnancy at 10-13 weeks of gestation. Cardiac defects (CD) were detected in 80.0% of all cases, in 78.8% of aneuploid fetuses and in 83.3% of fetuses with normal karyotype.

Karyotype	Total # of fetuses	# of fetuses with CD
Aneuploidy, including:	47	37
Trisomy 13	6	4
Trisomy 18	10	9
Trisomy 21	12	6
Other trisomies	2	1
Monosomy X	17	17
Normal karyotype	18	15
Total	65	52

These findings suggest that measurement of nuchal translucency thickness is effective for screening for cardiac abnormalities in fetuses with normal karyotype in addition to its role in detection of chromosomal disorders.

**Nature of a disease: Congenital deficiency of alpha fetoprotein.** *R. Sharony<sup>1</sup>, A. Amiel<sup>1</sup>, N. Bouaron<sup>1</sup>, D. Kidron<sup>2</sup>, D. Itzhaky<sup>3</sup>, M. Fejgin<sup>1</sup>.* 1) The Genetic Institute; 2) The Pathology Institute; 3) Immunology Laboratory, Sapir Medical Center-Meir Hospital, Kfar Saba, Israel.

The role of alpha-fetoprotein (AFP) is unknown for the most part. Several functions of AFP during fetal life have been suggested: regulation of osmotic pressure, mediation of the immune system and growth control. In this report we describe two cases of congenital absence of AFP that were identified by the current methods of detection. The pathological examination results, including an immunohistochemical stain, which refine the levels of AFP detected by the biochemical studies, are enclosed. In order to exclude chromosomal anomalies in the placentae, we performed complete genomic hybridization analysis on both placentae. The test did not reveal any abnormalities of the first placenta. However, the second placenta showed monosomy 16, which was confirmed by FISH. It has been reported that tissue-specific expression of the AFP gene is strongly stimulated by an enhancer present 3.3 to 4.9 kb upstream of the transcription initiation site mapped to 16q22.3-q23.1. An overview of the molecular biology of AFP production is set forth. An explanation is suggested for the lack of symptoms in a newborn of undetected levels of AFP and the mechanism by which this condition might occur. This may shed light on the mechanism by which this rare condition is generated and the lack of any symptoms in affected newborns.



**Testing Normality Of Fetal Dna Concentration In Maternal Plasma At 10-11 Completed Weeks' Gestation: Preliminary Approach To Integrated Screening For Aneuploidies.** P. Carinci<sup>1</sup>, E. Caramelli<sup>1</sup>, M. Concu<sup>1</sup>, N. Rizzo<sup>2</sup>, A. Farina<sup>1,2</sup> and Fondazione CARISBO" and "Progetto Giovani Ricercatori". 1) Dept Histology & Embryology, Univ Bologna, Bologna, Italy; 2) Department of Obstetrics & Gynecology, University of Bologna, Italy.

Extracellular foetal DNA has been detected recently in the plasma of pregnant women, and it could represent a further approach in non-invasive prenatal diagnosis. Aim of this paper was to evaluate the distribution of the values of foetal DNA in maternal plasma expressed as g/equiv of a population of normal pregnancies. Data were collected from 40 women who underwent a CVS. Samples were collected at 10-12 completed gestation weeks' determined by ultrasound scan. The blood samples, 5 ml, were processed within 24h of sampling. For analysis, DNA was extracted from 1 ml plasma (QiaAmp). The DNA extraction was carried out by female staff, to minimize the possibility of contamination. The DNA preparation were eluted in 200 ml elution buffer of which 10 ml was used as a template for the PCR reaction. To determine the amount of male foetal DNA the Y chromosome SRY locus was used. A standard protocol for Real time PCR was used. Descriptive statistics was performed by means of routine analysis. In order to tests for deviations from normal distribution, the Kolmogorov-Smirnov test (KS) was used. The KS statistic quantifies the discrepancy between the distribution of data and an ideal Gaussian distribution. The Dallal and Wilkinson approximation to Lilliefors' method was used. Maternal age was not taken into account in this paper as a possible confoundent on foetal DNA values, since our population was unbalanced. In fact 85% of the women were  $\geq 35$  years at delivery. Our results showed a normal distribution of fetal DNA at 10-12 week' gestation after natural logarithmic transformation. Mean value of foetal DNA was 71 g/equiv. This analysis can be considered a preliminary step for a possible integrated screening able to combine more variables to detect abnormal pregnancies and to improve sensitivity for genetic diseases at the first trimester.

**EVOLUTION OF THE DETECTION RATE OF CONGENITAL HEART DISEASES BY ROUTINE FETAL ULTRASONOGRAPHIC EXAMINATION.** *Y. Alembik, M.P. ROTH, B. DOTT, C. STOLL.* Service de Gntique Mdicale, Hpital de Hautepierre, Strasbourg, France.

The objectives of this study were to compare the detection rate of congenital heart diseases (CHD) by routine fetal ultrasonographic examination (US) from 1979 to 1999 in a well defined population. The material for this study came from our register of congenital anomalies which registered 13,500 births per year including livebirths, stillbirths and termination of pregnancy (TOP). Three US examinations, one for search of congenital anomalies and 2 for biometric purposes were performed routinely. CHD were classified as isolated or associated when at least one other major extra cardiac malformation was present. The studies periods were 1979 - 1988 (131,760 births), 1990 1993 (92,021 births) and 1994 1999 (80,076 births). For these 3 periods the overall detection rate (DR) was 9.2%, 13.7% and 19.9% respectively. The DR for isolated cases was 4.5%, 10.2% and 11.4% respectively. The DR for multiply malformed with CHD was 30.5%, 33.0% and 40.2% respectively. The DR for hypoplastic left heart was 38.7%, 46.1% and 64.3%, respectively, for tetralogy of Fallot 21.4%, 22.6% and 48.5%, respectively, for ventricular septal defect 4.3%, 6.6% and 19.8% and for atrial septal defect 5.8%, 8.8% and 7.2%. For all CHD, TOP was 2.6%, 6.4% and 12.3% respectively and for the CHD diagnosed prenatally TOP was 28.6%, 46.7% and 62.0%, respectively the results are in accordance with the other studies of routine prenatal diagnosis of CHD. However with the extended fetal ultrasonographic examination proposed by some authors the DR is much higher than in this study. In conclusion this study shows that there is an improvement in the prenatal detection rate of CHD over time but this increase is only slight, and higher for isolated cases than for multiply malformed with CHD. These results stress the need to obtain a definitive clear, four chamber view and a view of the inflow and outflow tracks of the fetal heart and to train sonographers in order to improve prenatal detection of CHD.

**PRENATAL DIAGNOSIS OF DYSMORPHIC SYNDROMES BY ROUTINE FETAL ULTRASONOGRAPHIC EXAMINATION ACROSS EUROPE.** *C. Stoll, M. Clementi.* Service de Gntique Mdicale, Hpital de Hautepierre, STRASBOURG, FRANCE.

The objectives of this study was to evaluate the prenatal diagnosis of dysmorphic syndromes by fetal ultrasonographic examination. Data from 20 registries of congenital malformations in 12 European countries were included in the study. 479 out of 2454 cases with congenital heart diseases were recognized syndromes including 375 chromosomal anomalies and 104 non chromosomal syndromes : 28 deletions 22q11 (44,4% were prenatally detected), 17 heterotaxy sequences (64,7% were prenatally detected), 15 VATER association (46,6% were prenatally diagnosed), and 44 others. 192 out of 1130 cases with renal anomalies were recognized syndromes, 161 out of them (83,3%) were diagnosed prenatally including 107 chromosomal aberrations and 64 non chromosomal syndromes (15 VATER, 13 Meckel Gruber, and 36 others). 54 out of the 250 cases with limb reduction deficiencies were recognized syndromes, including 16 chromosomal syndromes and 38 non chromosomal syndromes (7 amniotic bands, 6 VATER, and 25 others) ; 20 of them were diagnosed prenatally (37,0%) including 9 chromosomal syndromes. 57 out of 243 cases of abdominal wall defects were recognizable syndromes, 48 with omphalocele (27 chromosomal, 5 OEIS, and 9 others) and 9 with gastroschisis (4 limb body wall complexe, 2 megacystis-microcolon, 2 chromosomal aberrations and 1 OEIS), 48 out of them were diagnosed prenatally (82,5%). 64 out of 349 cases with intestinal anomalies were recognized syndromes, 34 were diagnosed prenatally (53.1%). There were 549 cleft lip and palate (CL(P)) and 197 cleft palate (CP) including 74 chromosomal aberrations and 73 recognised syndromes including 23 Pierre Robin. Prenatal diagnosis was done in 51 CL(P) (53.1%) and 7 CP (13.7%). Out of 290 cases with spina bifida, 18 were recognized syndromes, 17 of them were diagnosed prenatally. All 11 syndromic encephaloceles were diagnosed prenatally. In conclusion this study showed that around 50% of the recognized syndromes can be detected prenatally by the anomaly scan. However the detection rate varied with the type of syndromes and with the policy of prenatal screening between countries.

**Cornelia de Lange syndrome - Prenatal manifestations: Report of four cases.** *K.L. Chong<sup>1</sup>, M. Nowaczyk<sup>2</sup>, A. Pai<sup>1</sup>, P. Mohide<sup>2</sup>, D. Chitayat<sup>1</sup>.* 1) Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, Toronto, Ontario, Canada; 2) Department of Genetics, McMaster University, Hamilton, Ontario, Canada.

Cornelia de Lange (CdL) or Brachmann-de Lange syndrome is a complex disorder of unknown etiology usually characterized by severe mental retardation, prenatal and postnatal growth retardation, microcephaly, small stature, limb deformities and characteristic facial features. Most cases of CdL syndrome are sporadic; however, rare familial cases and association with duplications of chromosome 3q have been reported. We present here four cases of CdL syndrome detected prenatally and confirmed by autopsy/ postnatal examination to illustrate the salient features of this syndrome on detailed ultrasonographic evaluation. All cases had unilateral or bilateral upper limb abnormalities ranging from absent fingers to single phalanx to absent hand or forearms. Intrauterine growth retardation was not consistently present prenatally. The remaining fetal anatomy was usually normal but other anatomical findings were present in 1 case. All karyotypes were normal. All cases presented had characteristic facial features of CdL syndrome including high arched eyebrows, synophrys and micrognathia, which confirmed the diagnosis postnatally. Although the differential diagnosis for limb abnormalities is broad and should consider several syndromes including TAR, Holt-Oram, EEC, Roberts syndrome, and Fanconi anemia, these cases highlight the importance of postnatal examination in confirming the diagnosis of CdL syndrome when a fetus presents with phocomelia or limb/hand abnormalities.

**Prenatal diagnosis of Pallister-Killian syndrome in a fetus with increased nuchal translucency.** *K. Bakou<sup>1</sup>, P. Kaminopetros<sup>2</sup>, C. Sarri<sup>3</sup>, M.B. Petersen<sup>1,3</sup>, V. Velissariou<sup>1</sup>*. 1) Cytogenetics Laboratory, MITERA Matern. Surg. Center, Marousi, Greece; 2) Dept. Fetal Medicine, MITERA Matern. Surg. Center, Marousi, Greece; 3) Dept. Genetics, Inst. Child Health, Athens, Greece.

The case of a fetus with Pallister-Killian syndrome (tetrasomy 12p) is presented during prenatal diagnosis in the first trimester of pregnancy. Chorionic villus sampling (CVS) was performed during the 12th week of gestation because of increased nuchal translucency (3.1 mm), left diaphragmatic hernia, polydactyly and clinodactyly which were detected during routine ultrasound investigation. In the direct preparation, chromosome analysis showed a normal female karyotype 46,XX in a total of 17 cells. In the culture, an extra metacentric marker chromosome of unknown origin was detected in 19% of cells. G-banding was suggestive of an isochromosome i(12p). In order to distinguish between a case of true mosaicism or Confined Placental Mosaicism (CPM), amniocentesis was performed. The same marker chromosome was detected in 86% of the amniocytes. FISH analysis with a whole chromosome 12 paint probe confirmed that the marker was indeed an isochromosome i(12p) and that the fetus suffered from the Pallister-Killian syndrome. The parents elected termination of the pregnancy. The presence of the i(12p) chromosome was confirmed in skin fibroblasts of the aborted fetus.

**PRENATAL DIAGNOSIS AND FAMILIAL APPROACH OF A  $t(1;18)(q21.3;q11.2)$ .** *R. Baez-Reyes, G. Razo-Aguilera, R. Garcia-Cavazos.* Departamento de Genetica, Instituto Nacional de Perinatologia. MEXICO.

The balanced reciprocal translocation can produce disbalanced gametes that precipitate the loss of gestation or products with congenital defects, otherwise can block gametogenesis depending on the involved chromosome and the sex of the carrier. A case of couple that went to Prenatal Diagnosis consult is reported, a 32th-week pregnancy from last menstruation date, whose previous son had hidrocephaly and not specified structural defects. A high-resolution ultrasound is made reporting dilatation of posterior fossa, congenital cardiopathy, abnormal position of both hands and hydramnios. Cytogenetic amniocentesis is made, obtaining a chromosomic complement:  $47,XY,der(1)t(1;18)(q21.3;q11.2)+der(18)t(1;18)$  by GTG and FISH. Then, cytogenetic study is made to the parents, finding that the mother is carrier of the balanced reciprocal translocation, with chromosomic complement  $46;XX,t(1;18)(q21.3;q11.2)$ . A male newborn is obtained, who presents early neonatal death. Correlation phenotype-kariotype is made and the cytogenetic study is completed to 27 members of the family in order to give real genetic advice.

**Monochorionic twins with discordant sex in a triplet pregnancy.** *J. Claus*<sup>1</sup>, *R.A. Quintero*<sup>2</sup>, *B.G. Kousseff*<sup>1</sup>. 1) USF Regional Genetics Program, Tampa, FL; 2) Florida Institute for Fetal Diagnosis and Therapy, St. Joseph's Women's Hospital, Tampa, FL.

We report an IVF (with two-embryo transfer) triplet pregnancy in a 30-year old primigravida with sex discordant monochorionic diamniotic twins and fetal demise of triplet A. On targeted sonogram triplet B showed hydrops and evidence of a micropenis. Aspiration of the cystic hygroma for chromosome analysis and to provide space for the ligation of the umbilical cord was done. Endoscopy confirmed the presence of abnormal genitalia, suggestive of micropenis. The umbilical cord ligation was performed to prevent adverse effects of the spontaneous demise of triplet B on the health of triplet C. The karyotype was 45,X. Amniocentesis of triplet C showed 46, XY. Triplet C developed hydrops and a month later was miscarried at gestation 22 weeks. A pathology report described monochorionic triamniotic triplet placentation. DNA testing for zygoty was not performed. Sex discordance has been reported in twelve articles. Of these, two (Gonsoulin et al., *Prenat Diagn* 10: 25-8; Schmid et al., *Prenat Diagn* 1990 20:999-1003) were based on prenatal diagnosis. It is likely that the discordance was either due to postzygotic nondisjunction or anaphase lag, before or during twinning. This report emphasizes the fact that suspected monozygoty does not exclude karyotyping both sacs when anomalies are noted.

**Pragmatic implications (few normal embryos) of preimplantation genetic diagnosis (PGD) for chromosomal rearrangements.** *N.R. Agan<sup>1,2</sup>, S. Torsky<sup>1</sup>, P. Cisneros<sup>1</sup>, S.A. Carson<sup>1</sup>, J. Buster<sup>1</sup>, L. Swaim<sup>3</sup>, J.L. Simpson<sup>1,2</sup>, F.Z. Bischoff<sup>1</sup>.* 1) Departments of OB/GYN; 2) Molecular/Human Genetics, Baylor College of Medicine, Houston, TX; 3) Houston Women's Care Associates, Houston, TX.

Individuals with chromosome rearrangements are at an increased risk for abortions and children with birth defects. The 10-15% empiric risk of liveborn unbalanced segregants (adjacent; 3:1) can be addressed by PGD. However, the efficiency of PGD depends on how many normal/balanced embryos are present. **OBJECTIVE:** To determine the proportion of normal/balanced embryos in two couples with chromosomal rearrangements. **METHODS:** In couple A, the husband carries a balanced translocation [46,XY,t(5;13)(p10;q10)]; in couple B the wife a derivative chromosome [46,X,der(X)t(X;Y)(p22.13;q11.2)]. Single biopsied blastomeres from fertilized embryos were subjected to FISH analysis. **RESULTS:** Frequency of normal embryos is far less than the 85-90% expected at CVS/amniocentesis. **CONCLUSION:** In PGD for rearrangements, relatively few normal embryos are found. Programs must be prepared to screen many embryos; the number of embryos screened may be more than usually needed for routine IVF.

	Couple A-Cycle 1	Couple A-Cycle 2	Couple B-Cycle 1
# embryos biopsied	24	19	16
# abnormal embryos	17	15	11
# uninformative	4	1	0
# normal embryos	3 (12.5%)	3 (15.7%)	5 (31.25%)
Pregnancy	No	Pending	Yes



**Mosaic Trisomy 16: High Percentage of Trisomic Cells in Amniotic Fluid Followed By Favorable Outcome.** *K. Morris<sup>1</sup>, J.R.R. Batanian<sup>2</sup>, D.K. Grange<sup>1</sup>, D. Hoyt<sup>1</sup>.* 1) Pediatrics, St. Louis University, St Louis, MO; 2) Pediatrics and Pathology, St. Louis University.

Numerous cases of prenatally diagnosed mosaic trisomy 16 have been reported. Outcomes have been highly variable and few reports include information about health and development beyond the newborn period. We report a patient with mosaic trisomy 16 with a high percentage of trisomic cells in amniotic fluid, in whom the outcome has been good. Amniocentesis was done at 16 weeks gestation because a triple screen was positive for Down syndrome. Amniotic fluid chromosomes revealed 39 of 42 cells (93%) with a 47,XX,+16 karyotype. The pregnancy was marked by intrauterine growth retardation and premature delivery at 32 weeks. Blood chromosome analysis at birth showed 20/20 cells with a 46,XX karyotype while placental chromosome analysis showed 20/20 cells with a 47,XX,+16 karyotype. Skin biopsy showed 2/50 cells with a 47,XX,+16 karyotype. Maternal disomy 16 has been ruled out by blood studies. No serious medical problems have yet been identified in the child. She is being followed by cardiology for mild aortic valve stenosis, with a possible bicuspid aortic valve. Neonatal renal and brain ultrasounds were normal. Ophthalmologic abnormalities include cloudy corneas which cleared spontaneously, mild microphthalmia and "setting sun" sign. Bilateral inguinal hernias were repaired at 5 months. Dysmorphology exam after birth showed only a lowset, slightly dysplastic ear and slightly curved fourth toes. Repeat exam at 6 months of age showed plagiocephaly, prominent forehead and hemangiomas on the glabella and upper eyelids, but was otherwise normal. At 9 months chronological age, weight and head circumference were within normal limits for corrected age (25th and 50th%), while height was less than 5%. At 10 months (8 months corrected age), Denver assessment showed development in the 9-10 month range for all areas tested. Gross motor development was in the 10-11 month range. This case demonstrates that a favorable outcome in terms of health and development can occur even with a high percentage of trisomic cells in amniotic fluid, and with a low level of mosaicism postnatally.

**Quantification of all fetal nucleated cells in maternal blood of aneuploid pregnancies using FISH and PRINS techniques.** *K. Krabchi, M. Bronsard, J. Masse, J-C. Forest, R. Drouin.* Dept Med Biol, Laval Univ and Hosp St Francois d'Assise, CHUQ, Quebec, Qc, Canada.

Recently, we have demonstrated that it is possible to quantify the total number of all fetal nucleated cells per unit volume of maternal blood obtained between 18th and 22nd weeks gestation in normal pregnancies (Krabchi K. et al., Clin. Genet. 2001, in press). This number fluctuated between 2 to 6 fetal cells per mL of maternal blood. The purpose of our study is to quantify, using the same approach, the total number of fetal nucleated cells in maternal blood of pregnant women bearing an aneuploid conceptus. 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 technique (FISH) or PRimed In Situ (PRINS) labeling technique. Both techniques have been performed on blood specimens provided by eleven healthy pregnant women carrying an aneuploid fetus with the following karyotypes (the number of cases with this karyotype): 47,XY,+21(6); 47,XY,+18(1); 47,XXX(2); 47,XYY(1) and 69,XXX(1). Detection of fetal cells was carried out using specific sex chromosome probes directly coupled to fluorochromes for FISH ( CEP-X a-Satellite, CEP-Y Satellite III, LSI-21, CEP-18 a-Satellite, Vysis Inc.) or specific oligonucleotide primers for chromosomes X, Y, 21, 18, 7 and 8, followed by Taq DNA polymerase extension for PRINS reaction. The PRINS technique appears to be an interesting alternative to FISH and could be a useful tool for non-invasive prenatal cytogenetic analysis. Identification and quantification of fetal cells were performed by manual fluorescent microscopy screening. Between 11 to 26 fetal cells per mL were identified. These numbers are around 5-fold higher than the number of fetal cells observed in normal pregnancies. Thus, we demonstrate that it is possible, under optimized conditions, to reliably and reproducibly identify fetal cells in maternal blood, particularly when the fetus is aneuploid. In fact, in agreement with other studies, our results show that fetal cells in maternal circulation appear in much higher number in aneuploid pregnancies.

**Development of a new rapid test for Down's syndrome using real-time quantitative PCR.** *W. Holzgreve<sup>1</sup>, B. Zimmermann<sup>1</sup>, F. Wenzel<sup>2</sup>, S. Hahn<sup>1</sup>.* 1) Dept OB/GYN, Univ Basel, Basel, Switzerland; 2) Dep. Human Genetics, University Children's Hospital, Basel, Switzerland.

A significant proportion of clinical genetics is involved with the analysis of gross chromosomal anomalies. In prenatal diagnosis a major concern are aneuploidies, of which Down's syndrome is the most important in live births. The detection of these gross changes is still time consuming despite modern technologies such as FISH or quantitative fluorescent PCR. For this purpose we have developed a novel alternative using real time quantitative PCR using genetic loci in the Down's region of chromosome 21 and a control locus on chromosome 12. Simultaneous assessment of ratio of these two loci by multiplex real time PCR has shown that this technique can be used for the reliable and rapid distinction of trisomy 21 from karyotypically normal tissue. This technology can readily be extended to examine the most common fetal aneuploidies (13, 18, X and Y) or instances of chromosomal loss or gain. Furthermore, since it permits the rapid automatic analysis of numerous samples it is very well suited for high-throughput diagnostic settings.

**Prenatally diagnosed complex chromosome 4 rearrangement: A counseling dilemma.** *A.K. Yenamandra<sup>1</sup>, L. Mehta<sup>2</sup>, L. Hentze<sup>1</sup>, A. Shanmugham<sup>2</sup>, A. Ahmed<sup>3</sup>, P. Koduru<sup>1</sup>*. 1) Cell Genetics Lab, Dept. of Pathology; 2) Div. of Medical Genetics, Dept. of Pediatrics, North Shore University Hosp. Manhasset, NY; 3) Dept. Pathology, Univ. South Florida, Tampa, FL.

A 44 year old woman was referred for amniocentesis. She had a 4 year old healthy son and a history of two previous spontaneous abortions. Fetal karyotype was designated 46,XX,inv(4)(p15.2p16)(p12q21)(q33q35). Parental chromosomes were normal. FISH with coatosome 4 probe completely painted both homologues of chromosome 4. FISH with the Wolf-Hirschhorn region probe was normal in the fetus and the parents. Detailed fetal ultrasound and echocardiogram at 19 weeks gestation were normal. Parents were counseled that in a single de novo inversion involving two breakpoints, risks for abnormalities are approximately 7% (Warburton, 1991). The risk per additional breakpoint could be extrapolated to be 3.5% (Cotter, 1996), however this is arbitrary and in complex chromosome rearrangements (CCR) with several breakpoints information is scarce. Postnatal ascertainment of CCR's is biased towards high risks for mental retardation and physical defects. Based on the above, the risk of abnormality in this fetus was considered to be around 21%, given the six breakpoints. A normal fetal ultrasound and echocardiogram could indicate a lower risk. Despite the normal sonograms, the apparently balanced appearance of the rearrangement and normal FISH studies, the risk in this case was perceived as significant. The parents opted to terminate the pregnancy. Fetal examination could not be obtained. Multiple rearrangements within the same chromosome are rare, only one case of a double inversion of chromosome 5 presenting with cri-du-chat syndrome features could be ascertained. This case presented a counseling dilemma given the rarity of the abnormality and lack of information on prenatally diagnosed complex chromosome rearrangements.

**Advantage of utilization of whole genome amplification coupled comparative genomic hybridization in reproductive pathology.** *T.A. Loukianova<sup>1</sup>, S.S. Tang<sup>1</sup>, E. Rajcan-Separovic<sup>1,2</sup>, S. Ma<sup>3</sup>, D.K Kalousek<sup>1,2</sup>.* 1) Department of Pathology , University of British Columbia, Vancouver B.C; 2) Department of Pathology, Children's and Women's Health Centre, Vancouver, B.C; 3) Department of Gynecology, Vancouver General Hospital, Vancouver B.C.

Comparative genome hybridization (CGH) is a perfect tool for the recognition of aneuploidies that are common findings in reproductive pathology. However, the diagnostic ability of CGH is restricted by the amount of DNA required to perform CGH analysis. Maternal contamination also presents a problem for CGH analyses of reproductive tissues, originating from a pregnancy loss. Implementation of degenerate oligonucleotide-primed PCR(DOP-PCR) to supplement CGH technique expands the role of CGH in reproductive genetics. Utilizing DNA extracted from a small amount of tissue, even from a single cell represents, in addition, a sensitive diagnostic tool for detection of mosaicism in heterogeneous tissues. Our experience combining DOP-PCR with CGH comprises the analyses of 80 specimens including abortive tissues, gonadal tissues and tumors. All of them were successfully performed according to the published protocol by Kuukasjarvi et al. 1997. The template of DNA used for whole genome amplification ranged from 12pg (one cell extraction) to 200 ng. The amount of amplified DNA obtained was between 200-3000ng and allowed us to perform both CGH and SRY- specific PCR for sex confirmation. Cytogenetic findings include both whole as well as segmental chromosomal losses and gains. When possible, CGH results were verified with conventional cytogenetics. Our results highlight the advantage of the utilization of DOP-PCR coupled CGH in reproductive pathology.

**Improved MACS/CD71 fetal cell detection with rapid processing of maternal blood.** *J.L. Simpson<sup>1,2</sup>, D.A. Marquez-Do<sup>1</sup>, D.X. Dang<sup>1</sup>, C. Horne<sup>3</sup>, D.E. Lewis<sup>3</sup>, F.Z. Bischoff<sup>d</sup>*. 1) Depts of OB/GYN; 2) Molecular/Human Genetics; 3) Immunology, Baylor College of Medicine, Houston Texas.

Increasingly investigators are pursuing strategies for isolation and analysis of fetal cells in maternal blood as a method of noninvasive prenatal diagnosis. Given the low number of circulating fetal cells at any given time during pregnancy, optimizing methods for enrichment is necessary to maximize yield of these rare cells. **OBJECTIVE:** To determine whether the time at which sample processing is begun affects fetal cell detection. **METHODS:** Blood samples were obtained from women (n=25; ranging from 10.1 to 20.7 weeks gestation) prior to invasive prenatal genetic diagnostic procedure. Samples were blinded, divided into two aliquots, and processed within 2 or 16-18 hours later. Processing involved separation using discontinuous double gradient Percoll followed by MACS enrichment of CD71<sup>+</sup> cells. Recovered cells were analyzed by FISH for X and Y chromosomes. **RESULTS:** Frequency of fetal (XY) cells detected among the split specimens is summarized in the table below. Results were no influenced by gestational age.

Fetal Sex	Process within 2 hrs	Process within 16-18 hrs
Male	5/15 (33%)	2/15 (13%)
Female	1/10 (10%)	1/10 (10%)

**CONCLUSIONS:** A greater than two-fold increase in the detection of XY-cells observed in split samples processed within 2 hrs suggests that fetal CD71<sup>+</sup> cells may be less stable than maternal cells. Alternatively, these cells may be too low in number and variable among cases. Hence, an alternative marker (eg gamma globin) may be required for samples processed at a later time.

**Poor outcome in Down syndrome fetuses with cardiac anomalies or growth retardation.** *I.M.E. Frohn-Mulder<sup>1</sup>, M. Wessels<sup>2,3</sup>, F. Los<sup>2</sup>, P. Willems<sup>2</sup>, M. Niermeijer<sup>2</sup>, J. Wladimiroff<sup>2,3</sup>.* 1) Pediatric Cardiology, Sophia Children's Hospital, Rotterdam, The Netherlands; 2) Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands; 3) Department of Obstetrics and Gynecology, Univ Hospital Dijkzigt, Rotterdam, The Netherlands.

In some cases of fetal Down syndrome an ultrasound scan performed in the second or third trimester of gestation shows fetal abnormalities leading to a cytogenetic diagnosis. Genetic counseling in these pregnancies is difficult as the prognosis of this particular group of Down syndrome fetuses is not well known. To determine the outcome of these pregnancies, we studied 55 women with sonographically suspected fetal anomalies, polyhydramnios or growth retardation due to trisomy 21 in the fetus. A detailed scan was performed to determine the nature of the anomalies and possible associated malformations. Cardiac malformations were diagnosed pre- and postnatally in 29 out of these 55 Down fetuses (53 %), with complete or incomplete atrioventricular septal defects and ventricular septal defects being the most frequent anomalies. The most frequent non-cardiac findings were a relative short femur (45 %) and a small-for-gestational age (SGA) fetus (27 %). Termination of pregnancy was carried out in 25 out of 55 pregnancies (45 %). Of the 30 continuing pregnancies, 10 ended with intrauterine death whereas the remaining 20 pregnancies resulted in the delivery of a live-born infant at a mean gestational age of 37 weeks. The prognosis of these live-born Down infants was poor with a 1-year survival of only 60 %. Combining intrauterine death and death in the first year of life indicated that the overall survival rate was only 40 %. Fatal outcome was noted in 68 % (13/19) of the cases in the presence of congenital heart disease, 83 % (10/12) in SGA fetuses, 86 % (6/7) in combined congenital heart disease and SGA, but only in 17 % (1/6) in the absence of congenital heart disease and SGA. This study indicates that Down syndrome fetuses diagnosed in the second or third trimester have a poor outcome when they present with congenital heart disease and/or SGA. This is important in the genetic counseling of such pregnancies.

**Success rate for culture of fetal postmortem tissue is dependent on the method of pregnancy termination.** *E.J.T. Winsor<sup>1</sup>, R. Windrim<sup>2</sup>, G. Ryan<sup>2</sup>, G. Seaward<sup>2</sup>, D. Chitayat<sup>2</sup>, H. Akoury<sup>2</sup>.* 1) Pathology and Laboratory Medicine; 2) Obstetrics and Gynecology, Mount Sinai Hospital and University of Toronto, Toronto, Canada.

For pregnancies terminated because of fetal abnormalities, cell culture for chromosome analysis or molecular studies is extremely important in order to provide genetic counselling. Assessment of success rates for culture of fetal post mortem tissue is usually complicated by differences in tissue type, mixture of specimens from spontaneous demise and induced abortion and transport conditions. As part of a randomized controlled trial to evaluate methods of second trimester termination of pregnancy, fetal umbilical cord specimens were cultured from three patient groups: (1) intra-amniotic injection of prostaglandin F<sub>2a</sub> ; (2) vaginal misoprostol and (3) oral misoprostol. In this study umbilical cord specimens were uniformly collected in transport media and details of time of delivery and method of pregnancy termination were collected.

	IAPG (Group 1)	VM (Group 2)	OM (Group 3)	Total
Successful	37 (54.4%)	66 (90.4%)	39 (83.0%)	142
Failed	31 (45.6%)	7 (9.6%)	8 (17.0%)	46

There was a significantly higher success rate for culture in the vaginal and oral misoprostol routes when compared with the intra-amniotic injection of prostaglandin. Options for obtaining tissue for genetic testing should be discussed with the patient prior to pregnancy termination.



**Contribution of congenital anomalies to gestational wastage: a prospective study in a Brazilian University**

**Hospital.** *D.P. Cavalcanti*<sup>1,2</sup>, *A.R. Aguirre*<sup>2</sup>, *M.A. Pessoto*<sup>3</sup>, *M.F. Mello*<sup>4</sup>. 1) Dept Genetica Medica FCM, UNICAMP, Campinas, Sao Paulo, Brazil, MD, PhD; 2) Programa Genetica Perinatal; 3) Serv Neonatologia, MD; 4) Dept Anatomia Patologica, MD.

We design a prospective descriptive investigation at the Women's Hospital (Caism), to determine the precise contribution of congenital anomalies to gestational wastage. The study included fetus dying before delivery as well as that occurring in the early neonatal period. During 19 months (Sept/1999 to April/2001) we examined all perinatal deaths occurring in the obstetrical center of the Hospital. The study of the births followed a protocol including genetic-clinical examination, x-rays, clinical photographs, autopsy, and a cytogenetic study when necessary. In the referred period, we observed 5,757 births with an overall incidence of birth defects of 7.7%. From a total of 228 perinatal deaths (PD), 80 (35%) fetuses were malformed. The causes of gestational wastage were classified as maternal (85 - 37%), fetal (104 - 45%), or unknown (39 - 17%). Among PD from fetal causes, 24 (23%) cases were twins. The remaining cases were all malformed fetuses distributed as follows: 35 (44%) isolated defects, 15 (19%) fetuses with multiple anomalies, and 30 (37%) syndromic fetuses. In the isolated group, congenital malformations of the CNS (22) followed by urogenital (12) were the most frequent anomalies. Among syndromic cases, we found 15 chromosomal anomalies, 6 LBW/ADAM complex, 3 skeletal dysplasias, 3 lethal multiple pterygium syndrome and 1 Beckwith-Wiedemann syndrome. When the fetuses were subdivided in two groups, early gestational wastage (below 500g) and stillbirth / early neonatal deaths (500g or heavier), we observed among fetuses with a known cause of the death that fetal causes are more frequent in the later. The previously cited anomalies were found in both subgroups. With regard to all the known causes of death in the whole group, we observed that secondary prevention is possible in more than half of the deaths with maternal origin. Among deaths from congenital anomalies of the fetus, prevention by genetic counseling is possible in 81% of the cases. Financial Support FAPESP 98/16006-6.

**Amniotic fluid AFP values associated with fetal omphalocele.** *S. Diment, J. Roberson.* Department of Medical Genetics, Henry Ford Hospital, Detroit, MI.

The incidence of omphalocele has been reported as between 1 in 4,000 to 1 in 7,000 births. Approximately 50% of cases of omphalocele have other birth defects. 12-30% have chromosome abnormalities. Omphalocele is one of the ventral wall defects associated with elevated levels of maternal serum alpha-fetoprotein (MSAFP) and amniotic fluid alpha-fetoprotein (AFAFP). The median MSAFP for omphalocele in one series was 4.1 MOM with a range of 0.5-29.8 MOM. However, the median and range of AFAFP MOM for omphalocele has not been previously reported.

Between January 1987 - April 2001, the Henry Ford Hospital AFP Program received 30,812 amniotic fluid AFP samples. Of these, 41 were identified as confirmed cases of omphalocele. The AFAFP in these samples was between 0.23 and 71.08 MOM with a median of 2.46 MOM. (In gastroschisis the published median AFAFP MOM is 15.3). In our patients, 16 of 41 (39%) had a chromosome abnormality. Eleven had trisomy 18, three had trisomy 13, one had triploidy and one had an unbalanced translocation resulting in a duplication of chromosome 3q. The median AFAFP MOM in the chromosomally abnormal group was 4.13 compared to a MOM of 2.13 in those with normal chromosomes. Only 1 of 25 (4%) with normal karyotype had a positive acetylcholinesterase (ACHE) while 9 of 16 (56%) with an abnormal karyotype did. However, 3 of 16 (19%) of the chromosomally abnormal fetuses had an associated open spina bifida defect. In the 3 with omphalocele, open spina bifida and abnormal karyotype the median AFAFP MOM was 7.27 while in the 13 chromosomally abnormal fetuses without an open spina bifida defect the median MOM was 3.99. It is of interest that the medical literature indicates that fetuses with a chromosome abnormality tend to have smaller omphaloceles although in our series, AFAFP was higher in the chromosomally abnormal group.

**Maternal serum alpha-fetoprotein levels in pregnancies with fetal cleft lip and palate.** *S. Deering*<sup>1</sup>, *J. Bienstock*<sup>2</sup>, *K. Blakemore*<sup>2</sup>, *N. Hueppchen*<sup>1</sup>. 1) OB/GYN, National Naval Medical Center, Bethesda, MD; 2) OB/GYN, The Johns Hopkins University School of Medicine, Baltimore, MD.

**OBJECTIVE:** Elevated maternal serum alpha-fetoprotein (MSAFP) levels have been well-described in the literature in pregnancies complicated by midline ventral wall defects, such as gastroschisis or omphaloceles. There are a few case reports of patients with cleft lip/palate associated with elevated MSAFP values, but no large series. The objective of this study was to determine if MSAFP levels are elevated in pregnancies complicated by fetal cleft lip/palate. **STUDY DESIGN:** We conducted a review of the records from the previous decade of patients in our ultrasound database at the National Naval Medical Center as well as the records of patients followed in the cleft lip/palate clinic at the Walter Reed Army Medical Center. Additional data from similar patients were obtained from The Johns Hopkins Hospital database. For this study, we defined an elevated MSAFP level as greater than 2.5 multiples of the median (M.o.M.). **RESULTS:** We identified a total of 107 patients with pregnancies complicated by cleft lip/palate. Maternal alpha-fetoprotein levels were available for twenty of these patients. The mean MSAFP was 1.105 M.o.M. with a range of 0.415-1.981 M.o.M. **CONCLUSIONS:** Isolated fetal cleft lip/palate defects do not appear to be associated with an increase in maternal alpha-fetoprotein levels. Unlike other midline defects, which are known to cause an elevated MSAFP, the increased exposure of the nasopharyngeal tissue in cleft lip and palate patients does not appear to have the same effect.

**Complete absence of bile and pancreatic ducts in a newborn: a new entity of congenital anomaly in hepato-pancreatic development.** *K. Nakamura<sup>1</sup>, H. Mitsubuchi<sup>1</sup>, H. Miyayama<sup>2</sup>, J. Ishimatsu<sup>2</sup>, T. Yamamoto<sup>3</sup>, F. Endo<sup>1</sup>.* 1) Department of Pediatrics, Kumamoto University School of Medicine, Kumamoto, Japan; 2) Kumamoto City Hospital, Kumamoto, Japan; 3) Division of Molecular Pathology, Graduate School of Medical Science, Kumamoto University School of Medicine, Kumamoto, Japan.

Liver and pancreatic buds appear from the foregut at about 4 weeks of gestation in the human embryo. Each bud differentiates into tissue which consists of hepatocytes and bile duct epithelial cells in the liver and exocrine and endocrine cells and pancreatic duct epithelial cells in the pancreas. During the differentiation, each tissue requires a developmental process directed by intracellular signaling stimulated by extracellular factors such as cell to cell contact, extra cellular matrix and cytokines. Loss of one of these processes can lead to defects or to malfunctions of the organ and fetal death can occur. Here we examined a newborn infant who had no bile and pancreatic ducts. The girl had hydrops after 29 weeks of gestation and died shortly after birth. One of her siblings previously died with hydrops at about 6 months of gestation and there were 2 more miscarriages of unknown causes. The familial history suggested that there was a genetic cause of hydrops. The liver had an abnormally round shape and the pancreas was not present in the normal position. Although the number of islets was markedly reduced, there was an ectopic small pancreas with normally developed islets. Histological analysis revealed complete absence of extra and intra hepatic bile and pancreatic ducts. Liver and pancreas was infiltrated with white blood cells and tissue destruction was extensive. Immunostaining of these tissues showed albumin and alpha feto protein production but no positive bile duct marker staining such as CK19 in the liver. Insulin and glucagon staining in the remaining islets was positive. These clinical features have not been reported in the literature. These investigations suggest an existence of a gene linked to the development of both bile duct and pancreatic ducts.

**Folate, homocysteine, and vitamin B<sub>12</sub> in serum of mothers of children with nonsyndromic orofacial clefts. T.**

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There is a conclusive evidence that folic acid (FA) administered periconceptionally decreases risk for NTD. Periconceptional supplementation with FA has also been demonstrated to decrease a risk of nonsyndromic cleft lip and palate (NSCL/P) recurrences. If there is an insufficient level of folate available to cells, DNA synthesis and DNA methylation are decreased and homocysteine accumulates in blood of a mother and in amniotic fluid surrounding a fetus.

The purpose of this study was to compare serum levels of folate, B<sub>12</sub> and total homocysteine in mothers of children with NSCL/P and in control mothers.

**MATERIAL AND METHODS:** A case control design was used in this pilot study. In 22 case mothers and in 29 control mothers, detailed interviews focused on dietary and lifestyle patterns were conducted. Blood was drawn for analysis of serum total homocysteine, vitamin B<sub>12</sub> and folate.

**RESULTS:** In the sample of case mothers, the mean values were: total homocysteine - 11.6±4.9 mmol/l; vitamin B<sub>12</sub> - 291.3±127.3 pmol/l; folate - 15.6±5.4 nmol/l. In the sample of control mothers, the mean values were: total homocysteine 9.8±2.5 mmol/l; vitamin B<sub>12</sub>- 300.1±109.2 pmol/l; folate - 20.85.6 nmol/l. Thus, a highly significant difference was observed between case mothers and control mothers for serum folate level (P=0.0008), a moderate significance for total homocysteine (P=0.048), and no significance for vitamin B<sub>12</sub> (P=0.4).

**CONCLUSIONS:** Our preliminary data support a view that insufficient folate metabolism played a significant role in causing clefts in offspring of case mothers. This is corroborated by no dramatical differences in dietary patterns and nutritional values between cases and controls. We are aware that more precise evaluation of a possible role of hyperhomocysteinemia in etiology of clefts needs to be done by using an oral methionine loading test.

**The utility of magnetic resonance imaging (MRI) as an adjunct in the prenatal diagnosis of congenital anomalies.**

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Ultrasonography remains the imaging method of choice for the prenatal assessment of fetal anomalies due to its low cost, availability, safety and real-time capability. However, in some patients, clear images are not obtainable due to maternal obesity, fetal head engagement, oligohydramnios or other technical factors. Heretofore, the quality of conventional MRI was hindered by artifacts arising from the motion of the fetus. Recent technical advances in ultrafast MRI have enabled excellent resolution for fetal imaging by minimizing image degradation caused by fetal motion without the need for maternal or fetal sedation or paralysis. Compared with US, fast MRI has superior cerebral tissue contrast, operator independence, multiplanar imaging capacities and a larger field of view. Fast MRI is especially beneficial when evaluating fetal CNS abnormalities, particularly in detecting disorders of neuronal migration, evaluating the corpus callosum and posterior fossa, and in the evaluation of intrathoracic or intraabdominal anomalies. As with US, MRI does not expose patients to ionizing radiation, and there is no evidence that short-term exposure to electromagnetic fields harms the developing fetus.

We have recently performed ultrafast MR imaging on 7 pregnant patients referred for suspected fetal liver disease, familial microcephaly or ventriculomegaly. The results added invaluable clinical information that aided in genetic counseling and pregnancy management. Data from these cases and others from the literature further support the utility of fetal MRI as an adjunct to prenatal diagnosis in select cases.

**Limb-body wall complex in triplet pregnancy after IVF and CVS.** A.L. Shanske<sup>1</sup>, S. Pande<sup>2</sup>, K. Aref<sup>2</sup>, C. Vega-Rich<sup>2</sup>, S. Reznik<sup>3</sup>, I.E. Timor-Tritsch<sup>4</sup>. 1) Ctr. for Congenital Disorders; 2) Div. of Neonatology, and; 3) Dept. of Pathology, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY; 4) Dept. of Obstetrics, New York University School of Medicine, New York, NY.

The limb-body wall complex (LBWC) is a rare sporadic defect usually defined by the presence of at least two of three characteristics: limb defects, anterior body wall defects, exencephaly or encephalocele with or without facial clefts. The incidence of major malformations after IVF and embryo transfer is similar to that of the general population. Though clusters of limb defects have been reported after CVS, recent series have shown no difference from the background population. We have identified the LBWC in a triplet following IVF, embryo transfer and CVS.

A male infant weighing 1320 gm was delivered to a 38 year old mother after a 30 5/7 weeks triplet gestation by section. The mother required a hysterectomy because of placenta accreta. The pregnancy was established by implanting 4 frozen embryos. Three chorionic sacs with embryos were visualized at the time a CVS was carried out at 10 5/7 weeks. CVS was performed first on triplet A transvaginally and then on B transvaginally and transabdominally. No evidence of any fetal malformations were noted in the triplets and A and B were later noted to have normal karyotypes. A follow-up ultrasound at 12 4/7 weeks revealed in triplet B a non-membrane covered protrusion of small bowel to the right of the cord insertion.

Triplets A and C were normal. The newborn examination of B revealed an abdominal wall defect consisting of cloacal exstrophy with an omphalocele. The umbilical cord was 15 cm long, the left lower extremity was phocomelic with the foot attached directly to the thigh and there was a covered lumbosacral meningomyelocele. The infant expired the next day during the repair of the neural tube defect.

Limb defects have been reported with CVS and there has been a single report of LBWC after IVF. The pathogenesis of LBWC is controversial and probably heterogeneous. Vascular or membranous disruption is thought to play a role. It is possible that direct trauma to uterine vessels or fetal membranes contributed to the defects observed in our patient.

**The outcome and management of isolated prenatally diagnosed unilateral multicystic kidney.** *G. Aubertin<sup>1</sup>, S. Cripps<sup>2</sup>, G. Coleman<sup>2</sup>, D. Shaw<sup>3</sup>, L.T. Arbour<sup>1</sup>*. 1) Medical Genetics, UBC, Vancouver, BC, Canada; 2) Division of Urology, UBC, Vancouver, BC, Canada; 3) Division of Maternal Fetal Medicine, UBC, Vancouver, BC, Canada.

Unilateral multicystic kidney (UMCK) occurs in approximately 1 in 2500 live-births. With routine prenatal diagnosis, most will be detected prenatally. To delineate the natural history, clinical outcome and appropriate management of prenatally diagnosed isolated UMCK, we report our experience of 73 cases. Methods: All cases of prenatally detected isolated UMCK presenting at the BC Childrens and Womens Health Centre, from July 1987 to July 1998 were reviewed. A total of 73 cases met criteria for eligibility. Subsequently diagnosed birth defects, chromosome abnormalities, pregnancy complications, and family histories were reviewed. In addition, pediatric medical and surgical outcomes were available for 54 cases. Results: Of those with known post-natal outcomes, renal /genital-urinary tract abnormalities were diagnosed subsequently in 33%. Non-renal abnormalities were present in 16% of which congenital heart defects were most frequent (7%). One chromosome abnormality, a trisomy 21, was present among 32 cases (3%) where karyotypes were known. Amniotic fluid volume abnormalities were present in 11 cases but not predictive of the presence of birth defects with the exception of one case where polyhydramnios was associated with VATER Association. A family history of structural renal anomalies was reported in 20% of those with known histories. There were 14 partial or complete involutions (25%), including 2 complete prenatal involutions of the cystic kidneys. No long-term associated morbidity such as hypertension or malignancy was present in our cohort, however, our follow-up was limited to 11 years. Conclusion: Based on our study and corroborating literature, amniocentesis and fetal echocardiograms should be offered as part of prenatal care when a seemingly isolated UMCK is detected on routine prenatal ultrasound. A third trimester follow-up ultrasound, as well as careful assessment and follow-up of the newborn are indicated.



**The DNA-analysis in families with some neuromuscular diseases in Moldova, postnatal and prenatal diagnosis.** V. Sacara. National Centre of Medical Genetics, S R I C & M H C, Chisinau, Moldova.

The dystrophinopathies, Duchenne and Becker muscular dystrophy (D/BMD), have traditionally been considered two different diseases. It's became clear that these diseases represent different phenotypes resulting from different mutations in a single gene. Hereditary motor and sensory neuropathies (Chariot-Marie-Tooth Disease - CMTD). The most common cause of the disease is a duplication of 17p11.2-p.12. Spinal muscular atrophy (SMA)- clinical impression has been borne out by the mapping of all forms of spinal muscular atrophy to one small region of chromosome 5. In 99% of patients who had this disorder, a mutation was found in a gene called survival motor neuron (SMN). 81 families with increased risk of DMD, 15 families with SMA and 2 families with CMTD passed clinico-neurological, biochemical, muscle-ultrasonography investigations and molecular study. 64 out of 85 DMD patients (76%) were proved to be carriers of dystrophin deletion. 70.3% of deletions occupied the distal part of the gene. No obvious deletions were detected in 21 DMD patients and corresponding families were used for RFLP-analysis (pERT87-8/TaqI, pERT87-15/BamH and 16intron/TaqI polymorphism). RFLP-analysis was also applied to the deletion group which requested carrier detection. 41% of families referred for carrier detection were found to be informative for RFLP-analysis. In 2 cases performed preclinical B/DMD diagnosis. Molecular analysis was efficiently applied to 11 fetuses in MDD/B families. In 11 out of 15 SMA families have been established deletion of 7 exon and in 4 cases- deletion of 8 exon of SMN gene. In 4 cases we performed prenatal diagnosis SMA. The accuracy of PD in 2 fetuses informative for a deletion should be considered close to 100%. Molecular analysis was efficiently applied to 2 SMTD patients and was established duplications of 17p11.2-p12. Thus, thanking the conspicuous progress in molecular analysis of the different gene, possibility for higher efficiency of PD in D/BMD, SMA and SMTD families is quite evident. Deletion detection and carrier testing is a decisive step in elaborating a reliable strategy of prenatal diagnosis in families at high risk of DMD, SMA and SMTD.

**Monozygotic twinning rate after artificial reproduction technology.** *C.A. Derom<sup>1</sup>, R.M. Derom<sup>2</sup>, R.F. Vlietinck<sup>1</sup>*. 1) CME, Genetic Epidemiology, Leuven, Belgium; 2) Association for Scientific Research in Multiple Births, Belgium.

While more and more authors have no doubt that the monozygotic twinning frequency is increased in association with artificial reproduction technology (ART), many questions about this unexpected phenomenon remain unanswered. The magnitude of the increase varies substantially according to the nature of the samples studied and the type of ART (ovarian stimulation alone, IVF and related procedures, timing of the transfer etc.). Most of the studies in the literature rely on hospital-based data and on chorionicity rather than on zygosity. This latter may underestimate the incidence of zygotic splitting, as one third of pairs are dichorionic in spontaneous monozygotic twin pregnancies.

This study reports on the monozygotic twinning rate after different assisted reproductive treatment modalities and different drugs to induce the ovulation in a population-based registry of multiples with known zygosity and chorionicity. Since 1964 the East Flanders Prospective Twin Survey collects data on all the multiples born in the province of East-Flanders, Belgium. Between 1976 and 2000 1346 twin pairs and 127 triplet sets were born after ART. Basic perinatal data, placentation and zygosity were recorded at birth. Of the 1346 twin pairs 93% are dizygotic, 4.5% monozygotic and 2.5% dichorionic with unknown zygosity. Of the 127 triplet sets, 86% are trizygotic, 10% dizygotic, 1% monozygotic and 3% trichorionic with unknown zygosity.

Within the twin sample the ratio of monozygotic versus dizygotic twins differs substantially according to the drug used to induce the ovulation and the type of ART. When clomiphene alone was used as treatment this ratio is 11% as to compare to 3% when no clomiphene was used ( $p < 0.001$ ). After induction of ovulation without IVF this ratio is 7% as to compare to 3% after IVF ( $p < 0.001$ ). The same trends are seen within the triplet sample.

If confirmed by other studies, clomiphene could be the first identified drug influencing the monozygotic twinning rate in humans.

**Variations in termination rates in pregnancies diagnosed with Klinefelter syndrome: Data from a cross**

**European study.** *I. Nippert<sup>1</sup>, B. Eiben<sup>2</sup>, P. Miny<sup>3</sup>, T. Marteau<sup>4</sup>, C. Mansfield<sup>4</sup>, M. Reid<sup>5</sup>, M. van Diem<sup>6</sup>, S. Garcia Minaur<sup>7</sup>, D. Kirwan<sup>8</sup>, S. Walkinshaw<sup>8</sup>, C. Verschuuren-Bemelmans<sup>6</sup>, C. de Vigan<sup>9</sup>.* 1) UKM, Muenster, Germany; 2) Inst f Klin Genetik NR, Germany; 3) Univ-Kinderspital beider Basel, Switzerland; 4) Guy's, King's & St Thomas' Schools of Medicine, London, UK; 5) Univ of Glasgow, UK; 6) Rijksuniv Groningen, NL; 7) Hospital de Cruces, Barakaldo, Spain; 8) Women's Hospital, Liverpool, UK; 9) EUROCAT Paris, France.

Introduction: Klinefelter syndrome (KS) occurs appr in 1 per 800 male live births. About 10%-20% are identified by prenatal diagnosis (PD). Most of these cases are detected incidentally when PD has been performed because of an increased risk for Down syndrome. Because KS is likely to be unfamiliar, information given about KS may be of crucial importance for parents' decisions about whether or not to continue with the pregnancy. Methods: A systematic review of the case notes of all KS diagnosed up to 24 weeks of gestation in 8 European regions in 5 European countries was conducted. The variables documented included maternal age, parity, gestational age at diagnosis, speciality of health professional providing information before and after PD. Results: Details of 111 pregnancies and their outcome were obtained. 44.1% pregnancies were terminated. Across the 8 European regions termination rates varied between 76.9% and 0%. Using multivariable logistic regression analysis, the only significant predictor of continuation of the pregnancy was the speciality of the health professionals conducting post diagnosis counseling: the affected pregnancy was more likely to continue when post diagnosis counseling involved only a genetics specialist (RR 2.42 (95% CI 1.14-5.92)). Discussion: There is an association between whether or not a pregnancy affected by an unfamiliar fetal anomaly is terminated and the professional background of the health professional providing counseling. The causal nature of this association remains to be determined. Health professionals in charge of organizing and delivering information services in prenatal testing should be aware that the way information is provided may impact parents' decisions. Introduction  
Methods Results Discussion.

**Bickers-Adams phenotype caused by disruptive brain damage.** *F. Enza-Razavi<sup>1</sup>, P. Saugier-Veber<sup>2</sup>, J. Martinovic<sup>1</sup>, C. Carves<sup>2</sup>, M.C. Aubry<sup>3</sup>, J.M. Jouannic<sup>3</sup>, Y. Dumez<sup>3</sup>, M. Vekemans<sup>1</sup>, T. Frebourg<sup>2</sup>.* 1) Département de Génétique et Service d'Histo-Embryologie et de Cytogénétique, Hôpital Necker Enfants-Malades, Paris, France; 2) Service de Génétique, CHU de Rouen et INSERM EMI 9906, IFRMP, Faculté de Médecine et de Pharmacie, 76183 Rouen, France; 3) Maternité, Hôpital Necker Enfants-Malades, Paris, France.

Bickers-Adams syndrome, now referred as Hydrocephalus with Stenosis of the Aqueduct of Sylvius (HSAS), is characterized by a broad spectrum of clinical and neurological abnormalities with inter- and intrafamilial variability i.e. mental retardation, hydrocephalus, lower limb spasticity and adductus thumbs. Neuropathological findings are conclusive in HSAS and consist of narrowing of the aqueduct of Sylvius and hypoplasia/aplasia of pyramids (responsible for the spasticity), occasionally associated to agenesis of the corpus callosum (which may explain mental retardation). HSAS was mapped to Xq28 and, to date, more than 75 different mutations in the *L1CAM* gene, which encodes a neural adhesion molecule, have been described. Here we present a sporadic case of hydrocephalus with adductus thumbs, detected by ultrasounds in a male fetus of 33 weeks' gestation. Neuropathological examination, after pregnancy termination, revealed a post-hemorrhagic triventricular dilatation with aqueduct of Sylvius obliteration, associated to severe hypoxic-ischemic brain damage with capsula interna and corticospinal tracts atrophy. Screening of the entire coding region of the *L1CAM* gene, using enzymatic cleavage and direct sequencing, revealed no mutation. We concluded therefore that the Bickers-Adams phenotype may result from disruptive hypoxic-ischemic lesions, mimicking the presentation of HSAS related to *L1CAM* mutations.

**Prenatal screening for aneuploidy using maternal blood spots on filter paper in the first and second trimester.**

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Prenatal Down syndrome (DS) and open neural tube defects (ONTD) screening using multiple maternal serum markers is now well established in the second trimester (T2) of pregnancy. Enough data have become available to consider moving maternal screening into the first trimester (T1) combined with an ultrasound (US) measurement of the fetal nuchal translucency (NT). In collaboration with NTD Laboratories, Procrea began offering to women in Eastern Canada, T1 and T2 maternal screening for DS and ONTD using dried blood spots on filter paper in 1999. NT measurements were selectively offered in T1 and US dating in T2. From 10 3/7 to 13 6/7 weeks, PAPP-A and free b-hCG levels were coupled with fetal NT when available. Between 14 and 22 weeks, AFP and free b-hCG were assessed. Following positive screening results, couples were given the choice to follow up in a private or public clinical genetic setting. Between April 1999 and May 2001, 10181 pregnancies were monitored; 9169 had T1 (90%) and 1012 had T2. NT data were provided with 93% of T1 samples. In 47% of T2 samples, US dating was available. 25% of women were 35 years or older. There were 13 confirmed cases of DS, 12 in T1 and 1 in T2. In T1, 8/12 (67%) were at risk by blood markers alone, 6/10 (60%) by NT alone and 8/10 (80%) by combined blood/NT results. In the T2 case of DS, blood markers were negative. Using combined blood/NT data in T1, 14 other confirmed aneuploidies were found to be at risk; trisomy 18 in 3, trisomy 13 in 1, Turner syndrome in 4, triploidy in 6 and rare chromosomal anomalies in 3 more. 2 cases of ONTD were found at US in T1. One trisomy 18 and one ONTD were detected in T2. Our results agree with recent T1 screening data and support the case for Canadian women of all ages, including those over 35, to be offered combined T1 screening for DS in view of the significant number of other aneuploidies that will be detected as well.

**A Tale of Two Tails: Prenatal Diagnosis of Two Pseudotails.** *A. Toi<sup>1</sup>, H.M. Bedford<sup>2</sup>, B. Lo<sup>2</sup>, D. Chitayat<sup>1,2</sup>*. 1) Mount Sinai Hospital, Toronto, ON, Canada; 2) Division of Clinical & Metabolic Genetics, Hospital for Sick Children, Toronto, ON, Canada.

Human tails are rare malformations which may be divided into 2 categories: true tails and pseudotails. The term true tail should be restricted to the vestigial remnant of the embryonic tail. They may be composed of various tissues including adipose, connective, muscle, nerves and blood vessels. Pseudotails may be the end result of a variety of mechanisms and may occur anywhere along the spine. These may contain teratomatous elements including adipose tissue or cartilage. In both cases, spina bifida is the most commonly associated anomaly. Due to overlap of features, distinguishing a true tail from a pseudotail may be difficult.

We report 2 prenatally diagnosed fetuses with pseudotails. The first case was detected at 20 weeks gestation as a spinal appendage at the level of S3. Adjacent skin thickening with fatty echos in the spinal canal were seen in serial ultrasounds. A 3 cm finger-like appendage in the lumbar spinal region was confirmed at delivery. Neurologic examination was normal. No other anomalies were present. MRI of the spine confirmed a lipomyeloschisis, syringomyelia and a low-lying cord tethered to the fatty mass. The second case was detected at 19 weeks as a spinal projection at the level of S4. At delivery, a 5 cm curly tail-like appendage was confirmed in the L5 region originating from a base of thickened subcutaneous tissue. Stimulation of the appendage resulted in a further reflex curling. Neurologic examination was normal aside from an asymmetric anal reflex. MRI of the spine demonstrated an open dorsal sacral canal with extension of the fatty mass within the spinal canal, syringomyelia and a low-lying cord tethered to the mass. Unilateral duplication of the collecting system was seen on renal ultrasound. In both cases, good fetal movements were present and MS-AFP levels were in the normal range. Tails should be included in the differential diagnosis of prenatally detected spinal appendages and particular attention focused on the identification of an underlying spinal dysraphism. Families should be counselled about the potential for neurologic deficits.

**Prenatal detection and perinatal management of a giant cervical teratoma.** *L. Cartier, S. Khalife, V. Desilets, L. Beaumier, H. Flageole, J-M. Laberge.* McGill University Health Centre, Montreal, PQ., Canada.

A routine ultrasound at 18wks. revealed a left, multiloculated, cervical cystic mass. The differential diagnosis included; cystic hygroma, cystic teratoma, thyroglossal duct cyst, branchial cleft cyst, and hemangioma. Upon referral to our Fetal Diagnosis and Treatment Group, the patient met with the genetics team, a perinatologist, and a pediatric surgeon to discuss possible etiologies, testing indicated, prognosis, and pregnancy management options. The fetal karyotype result was 46, XX and a fetal echocardiogram was normal. Follow-up ultrasound findings were highly suggestive of a cervical teratoma measuring initially 35 x 42 x 43mm (32cc). Prenatal aspiration and amnioreduction were required on multiple occasions, removing from 300 to 800cc of cystic fluid and from 500 to 3,000cc of amniotic fluid at each sitting. An MRI was performed at 30wks. to evaluate the fetal airway. At 32.5 wks., with the mass measuring 17cm x 10cm x 7.5cm, cyst aspiration was performed (300cc), followed by the EXIT procedure to deliver the baby. The mass was resected, and pathology confirmed a teratoma. Its final weight was 1.1kg. and that of the baby 1.6kg. Postoperatively she has suffered from a left vocal cord palsy and gastroesophageal reflux necessitating a fundoplication. Discharged home at 3 months of age, she is doing well. The successful outcome is directly related to the quality of care given prenatally and postnatally to both mother and child. The couple was kept well-informed throughout, and was well supported by strict follow-up. The importance of referral to a multidisciplinary team of geneticists/ genetic counsellors, ultrasonographers, pediatric surgeons and neonatologists involved in fetal diagnosis and treatment, when a fetal malformation is diagnosed, cannot be overemphasized.

**Anhydramnios managed by serial amnioinfusions subsequent to failure of previously functional fetal vesico-amniotic shunt resulting in longterm perinatal survival.** *C. Kovac, B. Pierce, B.C. Calhoun, P. Nielsen, B. Joyner, R.F. Hume.* Madigan Army Med Ctr, Tacoma, WA.

Fetal obstructive uropathy with complete obstruction can lead to progressive oligohydramnios, fetal deformation, pulmonary hypoplasia, and renal dysplasia. Reported interventions include vesico-amniotic shunt placement in fetuses that meet eligibility criteria. Unfortunately, the clinical spectrum include cases which may challenge the medical-ethical decision making process.

Three recent cases involved late presentation of anhydramnios and cystic renal dysplasia. One case presented at 24 weeks with endstage polycystic kidney disease. A second case presented later with megacystis, and bilateral endstage renal dysplasia. Fetal karyotype obtained at bladder tap revealed 46 XY. The third case presented with severe oligohydramnios at 21 weeks gestation. After evaluation of fetal renal function with serial vesicocenteses, normal karyotype, and fetal therapy ethics board review, a vesico-amniotic shunt was placed. Amniotic fluid appeared stable for 7 days, after which the shunt appeared to migrate into the fetal bladder. In an attempt to prevent further pulmonary hypoplasia the remainder of the pregnancy was managed with serial (5) amnioinfusions between 22-28 weeks gestation. The patient subsequently developed chorioamnionitis and delivered a viable 1538 gm male infant at 29 weeks gestation. He initially required mechanical ventilation, but was extubated to room air at 12 hours of life. VCUG confirmed PUV and high grade bilateral vesicoureteral reflux. The infant went home at 12 weeks of age, with severe renal insufficiency, now requiring dialysis. Infant status remains guarded while awaiting renal transplant.

Antepartum amnioinfusion has traditionally been limited to evaluate fetuses with severe oligo/anhydramnios. Therapeutic amnioinfusion may prevent pulmonary hypoplasia and contractures. However, the risks are significant, including infection and preterm birth, and should be weighed against any potential benefit of this palliative treatment.



**Fluorescence In Situ Hybridization in uncultured amniotic fluid cells.** *V. Catala, E. Cuatrecasas, J. Colomer, M. Crespo, E. Dachs, T. Escabias, A. Seres-Santamaria.* Prenatal Genetics, Barcelona, Spainà.

Fluorescence in situ hybridization (FISH) is a technique than allows the evaluation of chromosome copy number in interphase cells. This characteristic gives FISH a great potential for the study of amniotic fluid samples for prenatal diagnosis. The major indications for prenatal diagnosis, such as advance maternal age, fetal malformation detected through ultrasonography, increased risk of trisomy estimated by maternal serum screening, or maternal anxiety require a rapid and accurate result. The classical cytogenetic analysis of amniotic fluid cells is, actually, the best technique to detect numerical and structural chromosome aberrations after cell culture, but the study is not completed in less than a week. FISH in uncultured amniotic fluid cells permits the detection of the most common aneuploidies in 24 hours after amniocentesis. We present a prospective study of 3047 uncultured AF samples by FISH, using commercial (ONCOR and Vysis) probes specific for 13, 18, 21, X and Y human chromosomes. FISH studies in amniotic fluid cells were performed in our laboratory since 1993 as an adjunctive of conventional cytogenetic studies. The characteristics of the probes (labelling, type of probe) have changed during the study. The best results were obtained using AneuVysion Assay (Vysis). Parallel cultures from each sample were grown and a standard cytogenetic result was informed at the end of the study. Sex chromosome aneuploidy was 0.7%. A total of 2% autosomal trisomies were observed Other authors have previously reported the use of FISH on uncultured amniotic fluid cells. Our results support than FISH is a feasible method to assess the most common aneuploidies. Actually FISH has to be considered, as an adjunctive method to classical karyotyping, however FISH allows an accurate result in less than 24h, and a rapid detection of aneuploidies is very important in prenatal diagnosis.

**Increased active GSTM1 (+/+; +/-) genotypes in endometriosis.** *D.A. Marquez-DO<sup>1</sup>, D.X. Dang<sup>1</sup>, M. Heard<sup>1</sup>, S.A. Carson<sup>1</sup>, J. Buster<sup>1</sup>, J.L. Simpson<sup>1,2</sup>, F.Z. Bischoff<sup>1</sup>.* 1) Depts of OB/GYN; 2) Molecular/Human Genetics, Baylor College of Medicine, Houston, TX.

Endometriosis affects 10-15% of premenopausal women. Although polygenic/ multifactorial inheritance is accepted, the underlying gene(s) and mechanism remain unknown. Given that environmental factors may be involved and given that the disease displays characteristics of malignancy, we are evaluating the role of polymorphic drug metabolizing enzymes in the pathogenesis of endometriosis. We postulate an association between the slow/rapid-acetylation NAT2 genotypes and/or the active/null GSTM1 genotypes among women with endometriosis. **METHODS:** Blood DNA from women surgically and histologically confirmed not to have endometriotic implants (n=37) or to have endometriosis was used for PCR amplification of the NAT2 (n=111) and GSTM1 (n=62) loci. For NAT2, restriction digestion was used to detect the wildtype (W) and mutant (M) alleles enabling classification into slow (M/M)- or rapid (W/W, W/M)-acetylation. For GSTM1, allele-specific PCR was used to distinguish active (+/- or +/+) from null (-/-) genotypes. **RESULTS:** See Table.

	NAT2/Slow	NAT2/Rapid	GSTM1 active	GSTM1 null
Endometriosis	45 (40%)	66 (60%)	49 (79%)	13 (21%)
Controls	15 (40%)	22 (60%)	16 (44%)	20 (56%)

**CONCLUSIONS:** We found an association between endometriosis and the GSTM1 active genotypes. The GSTM1 protein, which functions as a phase II foreign compound metabolizing enzyme, is believed to be important in the detoxification of the oxidative stress produced during the repair of the ovarian epithelium. We hypothesize that the active form may be involved in endometriosis through interactions with other cellular components which affect the expression of causative genes or protein binding.

**Prenatal diagnosis of Trisomy 12 Mosaicism: A Case Report and review of the literature.** *D.B. Schowalter, C. Zaleski.* Dept Medical Genetic Services, Marshfield Clinic, Marshfield, WI.

Mosaicism for trisomy 12 was ascertained prenatally through amniocentesis for maternal age. The small amount of published information surrounding trisomy 12 mosaicism makes prenatal counseling difficult. A 35-year-old Gravida I, Para 0 woman was referred for genetic counseling after amniocentesis results at 17 weeks gestation showed the presence of two cell lines. Sixteen colonies showed trisomy-12 and 10 colonies showed a normal female karyotype. These were all derived from three independent cultures. Level II ultrasound examinations performed at 19 and 27 weeks gestation revealed no structural abnormalities. Detailed pedigree was unremarkable other than a maternal cousin with Down Syndrome. This child's mother had a miscarriage and a stillbirth. At birth, there were no significant problems. Birth weight was at the 90th percentile, birth length over the 90th percentile, and head circumference was 75th percentile. Initial exam was unremarkable with the exception of a mongolian spot. Evaluation of the child by a clinical geneticist at 3 months was remarkable for slight posterior rotation of the ears, presacral dimple (base clearly seen), and the mongolian spot previously noted. Follow-up analysis of peripheral blood chromosome analysis was normal. One hundred cells were fully analyzed or scored for the presence of trisomy 12, and no trisomy cells were found in the study. The parents did not wish to pursue skin fibroblast chromosomes. We review the literature regarding prenatal mosaic trisomy 12, and outline the psycho-social issues the family dealt with throughout the pregnancy.

**Elevation of maternal serum hCG in the HIV+ women - implications for prenatal screening and placental**

**immunology.** *S. Klugman*<sup>1</sup>, *S. Carter*<sup>1</sup>, *B. Espinosa*<sup>2</sup>, *M. Crane*<sup>2</sup>, *C. Salafia*<sup>3</sup>, *S.J. Gross*<sup>1</sup>. 1) Obstetrics and Gynecology, Montefiore Med Ctr, Bronx, NY; 2) Bronx Lebanon Hosp, Bronx, NY; 3) Columbia U School of Public Health.

Ninety percent of women with human immune deficiency (HIV) infection are of reproductive age (NIH May 2001). Maternal multiple marker testing is now used routinely to screen for fetal NTDs and Down Syndrome. In a pilot study, we reviewed serum testing of HIV+ women who registered for care by the time of serum screening, and reported reduced mean human chorionic gonadotropin (hCG) in HIV+ women (Gross et al., ASHG #876;1997). The aims of this study were to validate our initial results and to identify underlying factors that may explain associations between maternal serum screening and HIV disease. Method: We reviewed the charts of pregnant HIV+ patients who had second trimester screening from 1994-2001. 44 of these patients had serum screening performed in the same laboratory. IRB approval for chart review of race, ethnicity, past reproductive history, viral load, CD4 count, concurrent illnesses, and serum screening results. Non-normal data was logtransformed prior to regression analysis with  $p < 0.05$  considered significant. Results: Both the mean hCG and mean AFP are increased (contrary to our pilot results). hCG levels were far more variable than the other markers. hCG MOM  $1.27 \pm 1.00$  (.44-5.2), AFP MOM  $1.52 \pm .8$  (.67-4.5). Increasing hCG MOM was highly correlated with decreasing CD4 count ( $p = 0.009$ ). Multivariate regression of effects of AFP and hCG on CD4 count showed only increasing hCG was independently associated with decreasing CD4 count ( $p = 0.02$ ,  $r = .44$ ). A non significant trend was found for increased hCG MOM with increasing viral load ( $p = 0.113$ ). Conclusion: Mean maternal serum MOM's for hCG and AFP are elevated in HIV+ women. HCG elevation is independently related to the patient's immune competence (as reflected by the CD4 count) and may result in an increased false positive rate for Down Syndrome risk assessment in this group. Furthermore, these findings may lead to further research and a better understanding of placental immune response and function in this setting.

**Prenatal Diagnosis in X-Linked Lymphoproliferative Disease.** *L. Li<sup>1</sup>, M. Morra<sup>2</sup>, C. Terhors<sup>2</sup>, J. Church<sup>1</sup>, J. Geng<sup>3</sup>, J. Zheng<sup>3</sup>, L. Cowan<sup>1</sup>.* 1) Dept. of Pediatrics, Childrens Hospital Los Angeles; 2) Div. of Immunology, Beth Israel Deaconess Med. Ctr., Harvard Medical School; 3) University Childrens Genetics Laboratory, ProGene Inc.

The discovery of genomic sequences and gene functions in human beings has brought about an enormous desire from the public for the application of basic scientific research in clinical medicine. Clinical molecular genetics laboratories have been taken to task to transform research achievement into clinical use, whereas quality controls in diagnosis are monitored by standard procedures and regulated by the College of American Pathologists (CAP). A recently cloned human gene, SAP (SLAM-associated protein), which plays an important role in regulating T/B-cell function against Epstein-barr virus (EBV) infection, was found defective in X-linked lymphoproliferative (XLP) disease. In the case reported here, the XLP mutation was detected in the proband by a research laboratory, and carrier testing and prenatal diagnosis were performed under the request of clinicians and family members at the University Children Genetics Laboratory, affiliated with Childrens Hospital Los Angeles. Using standard molecular techniques, the clinical genetic laboratory confirmed the carrier status of the mother and prenatal diagnosis on cultured amniocytes revealed that the male fetus inherited the XLP mutation. After consulting medical professionals and genetic counselors, the family decided to continue the pregnancy. A Follow-up study after the delivery confirmed that the babyboy has the same genotype in the XLP gene as his affected brother. The newborn baby was started on intravenous immunoglobulin infusions and anti-viral medicine at early stage, while bone marrow transplantation from an HLA-matched sister is planned to prevent EBV-related and unrelated complications. In conclusion, clinical genetic tests may not only predict the genotype of a fetus, but also help families and physicians to be prepared for early preventive treatment for affected patients with inherited disorders.

**Microchimerism for Y-chromosome bearing cells in nulligravid females presenting with pediatric autoimmune disease.** *R.N. Slotnick<sup>1</sup>, K. Atkins<sup>3</sup>, J. Leung<sup>1</sup>, A. Warren<sup>3</sup>, L. Blanchfield<sup>3</sup>, M.W. Stacey<sup>2</sup>, C. Gabriel<sup>1</sup>, C. Osgood<sup>2,3</sup>.* 1) Dept OB/GYN, Div Prenatal Diag, Eastern Virginia Medical Sch, Norfolk, VA; 2) Dept of Pediatrics, Eastern Virginia Medical School, Norfolk, VA; 3) Dept of Biological Sciences, Old Dominion University, Norfolk, VA.

Y-chromosome microchimerism within females has been suggested as a potential predictor, and possibly etiologically related to the development of autoimmune disease in women. Post-partum populations have been shown to carry low levels of fetal male cells, and in some cases microchimerism is detectable decades after delivery of a male infant. Autoimmune diseases have a higher prevalence in women as compared to men, including both rheumatologic and non-rheumatologic disorders. Female to male ratios of greater than 5:1 have been reported for systemic lupus erythmatosis (SLE) and scleroderma, while rheumatoid arthritis (RA) is at least three-times more common in females. Microchimeric cells have been identified in autoimmune disease lesions, lending credibility to the proposal that such cells may contribute to subsequent development of graft-versus-host disease in microchimeric females. Relatively little is known about the incidence or source of microchimeric cells in nulligravid females. We examined blood samples from 20 subjects, 10 diagnosed with SLE, 7 with RA and 3 with dermatomyositis. PCR primers from two regions on the Y-chromosome were used to detect qualitative presence of Y-bearing cells in DNA prepared from blood samples. In total, of 20 subjects, 70% showed detectable Y-chromosome DNA, while 30% did not. From 10 SLE patients, only one had a history of prior pregnancy, yet 80% of these patients were microchimeric. Of 7 nulligravid RA females, 57% were positive for Y-chromosome DNA. Two of three patients with dermatomyositis were positive for Y-chromosome DNA. Appropriate negative and positive controls behaved as expected. In summary, our results lend support to the proposal that nulligravid females with autoimmune disease are likely to carry chimeric male cells, and that such cells may contribute to disease development in those females.

**Second trimester screening for Down syndrome preferentially identifies affected pregnancies that are complicated by fetal hydrops.** *P.A. Benn<sup>1</sup>, J.F.X. Egan<sup>2</sup>, C.J. Ingardia<sup>3</sup>.* 1) Div Hum Genet, Dept Pediatrics, UCONN Health Ctr, Farmington, CT; 2) Div of MFM, Dept Ob/Gyn, UCONN Health Center, Farmington, CT; 3) Div of MFM, Dept Ob/Gyn, Hartford Hosp, Hartford, CT.

Low concentrations of second trimester maternal serum alpha-fetoprotein (MSAFP) and unconjugated estriol (uE3) together with high human chorionic gonadotropin (hCG) and inhibin-A (INH-A) are generally found in pregnancies complicated by Down syndrome (DS), hydropic Turner syndrome, or other non-immune hydrops. We compared the concentrations of these analytes in a group of 7 DS pregnancies with hydrops fetalis (generalized edema) and a control group of 85 DS pregnancies without hydrops. All cases were ascertained through positive maternal serum screening tests.

MS-AFP concentrations in the hydropic DS pregnancies (median 0.38, range 0.2-0.74 MoM) were significantly lower than those in the non-hydropic cases (median 0.82, range 0.37-1.86 MoM) (Mann Whitney test,  $P < 0.01$ ). Similarly, uE3 values were lower in hydropic (median 0.33, range 0.21-0.78 MoM) versus non-hydropic cases (median 0.63, range 0.16-1.16 MoM) ( $P < 0.01$ ). hCG levels were higher in the hydropic DS (median 4.07, range 3.28-10.36 MoM), relative to the non-hydropic pregnancies (median 2.51, range 0.76-6.86 MoM) ( $P < 0.01$ ). No significant difference was noted in INH-A concentrations for a sub-set of 5 hydropic cases (median 3.84 range 1.84-6.51 MoM) relative to that found in 42 non-hydropic cases (median 2.75, range 0.53-15.21 MoM). DS risks associated with these results were significantly higher for the hydropic cases ( $P < 0.01$ ) with all 7 hydropic cases having second trimester risks greater than 1:10.

These observations further strengthen the association between abnormal second trimester analyte values and disturbed fluid homeostasis. Increased nuchal thickness in DS fetuses probably represents a mild manifestation of fluid imbalance. A correlation may therefore exist between second trimester serum markers and ultrasound measurements of the nuchal fold.

**Pregnancy outcomes with undetectable uE3 on multiple marker screening.** *S. Dhandha, L. Fraer, W.A. Hogge.*  
Dept Genetics, Magee Hosp, Univ Pittsburgh, Pittsburgh, PA.

**Intro:** Undetectable unconjugated estriol (uE3) on multiple marker screen (MMS) has been associated with steroid sulfatase (STS) deficiency, fetal death, and IUGR. Our objective is to examine outcomes in pregnancies with undetectable uE3 on MMS, and to determine which MMS results are most likely to be associated with STS deficiency. **Methods:** We screened 70,018 patients between May 3, 1993 and April 30, 2001. Of these patients, 65 had undetectable uE3. We reviewed pregnancy outcomes in these cases, and correlated them with MMS results. **Results:** Of the 65 patients with undetectable uE3, 8 patients had an otherwise normal MMS. The 6 patients who were tested for the STS deletion were all carriers. Of the remaining 2 pregnancies, one was a pregnancy loss and one was a male at birth. 23 patients had an elevated AFP as well as an increased risk for trisomy 18 on MMS. An additional 4 pregnancies had only an increased AFP. All 27 resulted in pregnancy loss. 30 pregnancies had an increased risk for Down Syndrome and/or trisomy 18 on MMS. The 8 patients tested for the STS deletion were all carriers. Of the remaining cases, 4 had 46,XY karyotype. One patient declined amnio and STS testing, but had a family history of ichthyosis, and delivered a normal male. 14 of these pregnancies resulted in fetal losses, and 3 were lost to follow up. Excluding the losses, there were 6 pregnancies with an increased risk for Down Syndrome on MMS. All had an elevated hCG with a normal AFP. The 3 patients in this group tested for the STS deletion were all carriers. In contrast, there were 5 known surviving pregnancies among the patients who were screen positive for trisomy 18. All 5 had a low hCG and normal AFP on MMS. Four of these patients were tested and found to be carriers of the STS deletion. **Conclusion:** Patients with undetectable uE3 and normal MMS are all carriers of the STS deletion. Undetectable uE3 in association with elevated AFP is a marker of impending fetal demise. Patients with undetectable uE3 and an elevated hCG (increased risk for Down Syndrome) as well as those with undetectable uE3 and low hCG (increased risk for trisomy 18) should be offered STS testing.



**Outcomes in morbidly obese pregnant women.** *R. Dhanireddy, K. Yanamandra, T.F. Thurmon, D.D. Jefferson, C.S. Hill, D. Napper, J.A. Bocchini Jr.* Pediatrics, LSU Medical School, Shreveport, LA.

Our Pediatric Prenatal laboratory has been screening pregnant women from Northwest Louisiana for Neural tube defects (NTD) and Chromosomal Defects (CD) using triple screening (MSAFP, MShCG, and MSuE3) for the past several years. We routinely adjust MSAFP values for maternal weight, race and Insulin Dependent Diabetes Mellitus (IDDM) to improve the screening for NTD. After adjusting MShCG and MSuE3 values for maternal weight along with MSAFP and maternal age, prenatal CD risks are obtained. The reason for the maternal weight adjustment is to correct the analyte dilution from small or heavier mothers, so that precise prenatal risks are obtained. There have been several reports regarding the relationship of maternal overweight and susceptibility to birth defects including NTD. Thus we set out to investigate our prenatal screening by studying the delivery outcomes for any congenital anomalies. While analyzing the outcome data for NTD and CD for maternal weights in excess of 300lbs (300lbs-415lbs) and eliminating the possible confounding problems, we have not found any congenital anomalies. However, we have found that a statistically significant proportion of them have been delivered by Ceasarean section, 47% vs 20% in total population (OR 3.6, 95%CI 2.0-6.3). On stratification of the data by ethnicity, we found that both African-American and the Caucasian population are at higher risk. It is highly advisable to have genetic and obstetric counseling with these patients to apprise them of possible obtetric complications and the need for constant monitoring of their pregnancies. Data and statistics including the composition of outcomes will be presented.

**Activin A levels in pregnancies affected with neural tube defects.** *G. Lambert-Messerlian*<sup>1</sup>, *P. Florio*<sup>2</sup>, *F. Petraglia*<sup>2</sup>, *F. Severi*<sup>2</sup>, *J. Canick*<sup>1</sup>. 1) Pathology and Laboratory Med, Women & Infants Hosp, Providence, RI; 2) Obstetrics and Gynecology, University of Siena, Italy.

Activin, a member of a family of dimeric proteins originally identified in the reproductive system, has now been localized to many non-reproductive tissues and is a growth factor in a diverse range of cell systems. In humans, activin A is detected in amniotic and extraembryonic coelomic fluids and in maternal and fetal serum. Furthermore, activin b subunits and receptors have been colocalized in human fetal spinal cord and cerebrum. Taken together, these results suggest a role for activin in the development of the human nervous system. We hypothesized that activin levels may be altered in pregnancies affected by fetal neural tube defects (NTD).

Nine maternal serum (MS; 5 spina bifida, 4 anencephaly) and six amniotic fluid (AF) samples (4 spina bifida, 2 anencephaly), collected and identified through Women & Infants' prenatal serum screening program and at the University of Siena, were retrieved from freezer storage. Five unaffected samples were matched to each case for same completed week of gestation and approximate date of freezer storage. Total activin A levels were measured by Serotec assay (Oxford, UK) without operator knowledge of sample identity. The median activin A level in MS was 1.80 ng/mL (range 0.66-4.33) in unaffected pregnancy and 2.21 (0.68-3.51) in cases of NTD. The median activin A level in AF was 9.53 ng/mL (3.03-23.56) in unaffecteds and 12.03 (6.48-25.45) in cases of NTD. Activin A levels increased in unaffected MS (12% per week during 15-18 weeks gestation) and AF (18% per week during 17-22 weeks). After data were normalized for gestational age effects, levels of activin in MS from cases of NTD were not different from matched controls (median = 1.19 MoM,  $c^2 = 1.61$ ,  $p = 0.20$ ). However, levels of activin A in AF from cases of NTD were 44% higher (1.44 MoM,  $c^2 = 3.7$ ,  $p = 0.06$ ) than controls. Further studies are warranted to determine whether the level of activin A in amniotic fluid is a marker of NTD and to establish whether activin has a role in the development of the human nervous system.

**First Trimester Down Syndrome Screening in Twins: Moving From "Pseudo-Risk" to Real Risk.** *D.A. Krantz<sup>1</sup>, T.W. Hallahan<sup>1</sup>, E. Orlandi<sup>2</sup>, C. Rossi<sup>2</sup>, J.W. Larsen<sup>3</sup>, P.D. Buchanan<sup>4</sup>, V. Klein<sup>5</sup>, J.N. Macri<sup>1</sup>.* 1) NTD Laboratories, Huntington Stat, NY; 2) Centro di Diagnosi Prenatale, Palermo, Italy; 3) The George Washington Univ. Medical Center, Washington, DC; 4) GeneCare Medical Genetics Center, Chapel Hill, NC; 5) North Shore Univ., Manhasset, NY.

**Objective:** To develop a first-trimester Down syndrome screening method for twin pregnancies that factors in chorionicity along with the two nuchal translucency values and the free beta hCG and PAPP-A levels to calculate risks for each individual twin fetus, the risk that at least 1 twin might be affected and the risk that both twins might be affected and to determine if biochemical markers are necessary for this method. **Methods:** A series of 212 unaffected twin fetuses were used to develop reference data for the method. A Monte Carlo simulation of 100,000 sets of unaffected, discordant for Down syndrome and concordant for Down syndrome twins was performed. **Results:** In monozygotic pregnancies, at a fixed 5% false positive rate, the detection rate increased from 84% to 94% when biochemistry was included in addition to nuchal translucency while at a fixed 80% detection rate, the false positive rate was reduced from 3.0% to 0.5% by adding in biochemistry. In dichorionic pregnancies, in a population in which 2/3 of twins were dizygotic, the detection rate increased from 78% to 83% for discordant cases of Down syndrome and from 82% to 92% in concordant cases of Down syndrome when biochemistry was included in addition to nuchal translucency while at a fixed 80% detection rate for discordant cases, the false positive rate was reduced from 6.4% to 3.5% after adding in biochemistry. Results were similar for populations with a different percentage of twins that were dizygotic. **Conclusions:** This new method of screening for Down syndrome in twin pregnancy provides clinicians and patients with additional information compared to the current "pseudo-risk" screening method. Based on the data in this study the method is effective and the use of biochemistry can increase detection and/or reduce false positives compared to ultrasound alone.

**INTEGRATED PRENATAL SCREENING IN ONTARIO, CANADA.** *A.M. Summers, C. Meier, I. Lasis, P.R. Wyatt.* Genetics Program, North York General Hosp, Toronto, ON., Canada.

In 1999, Wald et al. described Integrated Prenatal Screening (IPS) for Down syndrome. IPS combined first trimester nuchal translucency (NT) and pregnancy associated plasma protein-A (PAPP-A) with triple or quadruple marker screening in the second trimester (*New Engl J Med* 341(7):461-7). IPS was expected to detect 85% of Down syndrome cases with a 1.5% positive rate if triple screening is used in the second trimester. This method of screening is dependent upon the woman receiving a single risk result at the end of screening in the second trimester. The purpose of this study was to confirm the estimated positive rate of IPS in a Canadian population and to assess feasibility and acceptability.

IPS was offered to women previously eligible for triple marker screening in the second trimester who presented before 13w6d gestation with a viable pregnancy. Screen positive women (risk > 1:200) were offered genetic counselling and amniocentesis with fluorescent in situ hybridization for chromosomes 13, 18 and 21 as well as full karyotyping. All women were offered a second trimester detailed ultrasound. Pregnancy outcomes are being obtained through hospital records.

Since November 1999, 2171 women have started IPS, 1875 women have completed 5 marker testing and 71 were screen positive. This gives a positive rate of 3.8%. Of the 71 positives, seven have been true positives for Down syndrome. The expected positive rate for IPS in this population (mean age = 32.3 yrs) would be ~3%. If this population of women had chosen triple marker screening, the positive rate would have been 15.8%. Results show that IPS performs as expected in terms of positive rate, and in our population, it is both feasible and acceptable.

**Maternal urine Invasive Trophoblastic Antigen (ITA) is a useful marker for Down Syndrome detection in the 1st Trimester.** *C.M. Strom<sup>1</sup>, G.E. Palomaki<sup>2</sup>, G.J. Knight<sup>2</sup>, L. Cole<sup>3</sup>, J.E. Lee<sup>1</sup>, R. Pandian<sup>1</sup>.* 1) Quest Diagnostics, San Juan, Capist, CA; 2) Foundation for Blood Research, Scarborough, ME; 3) U of New Mexico, Santa Fe, NM.

**Introduction:** Urinary ITA (u-ITA), a form of hyperglycosylated hCG has been reported to be elevated in Down Syndrome (DS) pregnancies in the 2nd trimester. We examined the utility of measuring u-ITA, urine total hCG (u-hCG) and urine beta core hCG (u-core) in the 1st trimester, either alone or in combination with established serum markers.

**Study Design:** A case-control set was created from specimens collected in a multicenter study of 4412 women having CVS or early amniocentesis at 9-15 weeks gestation (Haddow et al, NEJM 1998;338:955-61). Women with abnormal serum screens were excluded. Urine and serum samples were available from 17 DS pregnancies, and each case was matched with 5 controls. Urine was analyzed for u-ITA, u-core, u-hCG and normalized for creatinine. Serum values for total hCG (s-hCG), pregnancy associated plasma protein A (s-PAPP-A) and free beta hCG (s-beta) for these pregnancies were used for multivariate analyses.

**Results:** Univariately, the median u-ITA level in the 1st trimester was 3.16 MOM as compared to 2.52 MOM for u-hCG and 1.50 MOM for u-core. The observed detection rate (DR) for u-ITA at a false positive (FP) rate of 5% and 10% was 25% and 53% respectively, similar to that of u-hCG (24% and 53%) and much better than u-core (6% and 24%). Multivariately, the most powerful combination of 3 analytes was u-ITA with s-PAPP-A and s-beta yielding a DR of 74% and 81% at FP rates of 3% and 5% respectively. The DR is reduced from 81% to 74% if u-ITA is replaced by u-hCG.

**Conclusions:** Urinary ITA combined with s-beta and s-PAPP-A provides 1st trimester screening performance comparable to that obtained by 2nd trimester screening with 4 analytes (AFP, uE3,hCG,and dimeric inhibin A).

**A case study of prenatal phenotype of 48,XXXYY with elevated Maternal Serum Alpha-fetoprotein.** *B. Reddy<sup>1</sup>, S. Reddy<sup>2</sup>*. 1) MSAFP, Natl Medical Diagnostic Lab, Livonia, MI; 2) MGR Medical and Research Institute, Chennai, India.

It is a well documented fact that elevated maternal serum alpha-fetoprotein (MSAFP) is associated with various fetal abnormalities such as neural tube defects, ventral wall defects which affect intestines and other internal organs, congenital nephrosis, duodenal atresia, 48,XXYY syndrome and other abnormalities. In the present study we report a case of fetus with 48,XXXYY karyotype with elevated MSAFP. A 41 year old Asian Indian woman was referred for genetic counselling for advanced maternal age and consanguinity (marriage to 1st cousin). At 18 weeks of gestation MSAFP was elevated (3.32MOM). Pregnancy was terminated and post-mortem examination revealed a coarse facies with flat nose, epicanthic folds, low-set ears, VSD, clinodactyly of fifth fingers, kyphosis and proximal radio-ulnar synostosis. It has been previously reported that a prenatal phenotype of 48,XXYY was associated with elevated MSAFP. In the present report chromosomal analysis revealed a 48,XXXYY karyotype. It is not clear that how these chromosomal abnormalities are linked to elevated MSAFP. The role of consanguinity and various other plausible mechanisms involved in the elevated MSAFP are discussed.

**Simple and effective approach for detecting maternal cell contamination in molecular prenatal diagnosis. T.**

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The presence of maternal cells in prenatal tissues is an important issue, as a serious potential source for prenatal misdiagnosis. Maternal cell contamination (MCC) is increased with placental penetration and operator inexperience. This study presents our approach for detecting MCC at prenatal diagnoses. We performed 112 prenatal diagnoses: 79 cases for b-thalassemia/sickle cell anemia (62CVS/17AF), 14 cases for cystic fibrosis (1CVS/13AF), 10 cases for achondroplasia (all AF) and 9 cases for prelingual deafness (4CVS/5AF). The potential MCC in the prenatal tissues was determined by screening for MCC only the fetuses found to be carriers of the maternal mutation after the initial mutation screening, in all cases, except those of achondroplasia, which as a dominant or de novo condition can be misdiagnosed due to MCC. DNA samples from both parents were obtained before the amniocentesis. From the 112 cases 45 were finally tested for MCC (26CVS/19AF). The 3-HVR/APO B and the D1S80 loci were tested because of their high heterozygosity and the simplicity of horizontal agarose electrophoresis. 2 of the 25 cases tested for the 3-HVR/APO B and 4 of the 22 cases tested for the D1S80 were not informative. 3 additional loci were also used: the VNTRI of the vWFI gene (6 cases, all informative), the THO1 (6 cases, 1 not informative) and the TPOX (1 case, informative). In 4 cases of CVS where MCC was detected, DNA obtained from CVS culture was subsequently tested for MCC; if results were negative (2 cases), mutation screening was repeated and no amniocentesis was done. In the cases of confirmation of MCC in the cultured cells, amniocentesis was performed for further testing. In conclusion, testing of one locus presented sufficient information to exclude MCC for more than 90% of the cases. The choice of the appropriate locus is thereby essential, while the simultaneous screening of both parents provides for distinguishing the non-informative sites from MCC. The resultfulness, the low cost, the simplicity and the speed make this approach applicable in every diagnostic laboratory.

**Performance characteristics of a second generation genomic micro-array for the detection of single copy gains and losses.** *S. Stegenga, G. Browne, H. Burkhardt, M. Bushen, M. Hackbarth, T. Halder, M. Lucas, H. Marble, R. Miller, J. Piper, J. Proffitt, T. Ruffalo, J. Schultz, S. Seelig, J. Tomisek, K. Wilber, S. Willie, W. King.* Vysis, Inc., Downers Grove, IL.

We have developed a second-generation genomic micro-array system with the capability of detecting single copy changes by comparative genomic hybridization technology. The potential clinical applications for the genomic microarray include comprehensive prenatal and postnatal testing for microdeletion syndromes, subtelomeric deletions, aneuploidies and unbalanced translocations in a single microarray assay. The specimen types targeted for this product include amniocytes, chorionic villi and peripheral blood cells. This array contains approximately 750-1000 elements spotted on a chrome-plated chip surface. The genetic loci include clones covering 41 subtelomeric regions, common microdeletion syndrome regions, tumor suppressor genes as well as oncogenes that are amplified in a variety of cancers. To ensure a high quality product for potential clinical applications, hybridizations to several test DNAs containing known chromosome copy number changes as well as classical microdeletion syndromes have been used to validate the sensitivity and performance of the micro-array. Data on additional quality measures will also be presented.



**Incomplete methylation of the inactivated X-chromosome in human chorionic villous samples..** *M.S. Penaherrera, H.L. McDonald, C.J. Brown, W.P. Robinson.* Dept. Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

While X-chromosome inactivation (XCI) in somatic tissues is stably maintained and associated with methylation of most X-linked genes, the situation in placental tissues is less clear. Recent studies of XCI using a PCR based assay to examine methylation at the androgen receptor (*AR*) locus, have found evidence of lack of methylation for this gene in the stromal component of the placental chorionic villi. In an effort to investigate this issue, XCI status for several X-linked genes was evaluated in DNA extracted from 17 samples of cultured chorionic villi (CV) and 6 cord blood samples from normal female pregnancies. Five genes (*AR*, *FMRI*, *ARAF-1*, *MAOA* and *XIST*) were tested using methylation sensitive enzyme-based assays. Our results showed lack of methylation, demonstrated by failure to detect any PCR product after *HpaII* digestion, in 4/16 CV samples analyzed for the *AR* gene, 14/15 samples analyzed for *FMRI*, 15/15 samples analyzed for *ARAF-1* and 14/14 samples analyzed for *MAOA*. Lack of methylation was not observed in any of the 9 CV samples analyzed for the *XIST* gene. In contrast methylation was observed for all 5 genes analyzed using the 6 DNA samples extracted from cord blood.

These results demonstrate that lack of methylation is commonly observed for X-linked genes in human chorionic villous stroma. We hypothesize that genes located distant to the X-inactivation center may be more likely to lack methylation, and will test this by examining more genes. The significance of the hypomethylation to expression of X-linked genes is currently unknown. It is known that reactivation of X-linked genes occurs in extra-embryonic tissues and not in somatic tissues. However, the frequency of loss of methylation seen in the extra-embryonic tissue is surprisingly high if it reflects loss of silencing. We are therefore also investigating whether the methylation status is correlated with gene expression on the X-chromosome. The crucial role of the placenta in embryonic and fetal growth and development and the extensive use of CV sampling in prenatal diagnosis, highlights the relevance of understanding the mechanism of such a fundamental process as XCI in this organ.

**Prenatal and Preimplantation Genetic Diagnosis (PGD) for carriers of mtDNA mutations.** *H.J.M. Smeets<sup>1</sup>, J.G. Nijland<sup>1</sup>, L.J.A.M. Jacobs<sup>1</sup>, R.J.H. Galjaard<sup>2</sup>, H.R. Scholte<sup>2</sup>, H.F.M. Busch<sup>2</sup>, C.E.M. De Die<sup>1</sup>, J.P.M. Geraedts<sup>1</sup>, I.F.M. De Coo<sup>2</sup>.* 1) Dept Genetics and Cell Biology, Univ Maastricht, Maastricht, Netherlands; 2) Depts Child Neurology, Biochemistry and Clinical Genetics, Erasmus University, Rotterdam.

Options for carriers of mtDNA mutations to prevent transmission of these mutations to their offspring are limited. This is a major problem as it often concerns severe disorders with high recurrence risks. Recently, we screened the entire mtDNA of a patient with Leigh disease and identified the mtDNA T9176C mutation. Two other affected sibs carried the same mutation and mutation percentages varied between 93% and 97%. Their pregnant cousin also carried the mutation (55% in blood) and opted for prenatal diagnosis. Three criteria have been proposed for reliable prenatal investigations of mtDNA mutations: a close correlation between the proportion of mutant:wild-type mtDNA and disease severity; a uniform distribution of mutant mtDNA in all tissues; no change in mutant load in time. Based on existing, though limited data, the T9176C fulfils these criteria and the cut-off point for severe disease is about 90%. Prenatal investigations were performed on DNA from chorionic villus and amniotic cells and about 88% mutant mtDNA was detected in these tissues. Although the risk of a severely affected child was considerably, the pregnancy was continued. This family and data from literature illustrate that especially carriers with a high percentage mutated mtDNA are at risk of getting affected children. However, given the genetic bottleneck for mtDNA, these carriers may produce oocytes with low or absent amounts of mutant mtDNA. Therefore, we developed PGD protocols to select for embryos lacking mutated mtDNA. These protocols include the T8993G/C (NARP/Leigh), the T9176C (Leigh) and the A8344G (MERRF) mutation, but not the unpredictable A3243G (MELAS) mutation.

**Identification of alterations in the Huntington's gene using single cell PCR and the WAVE® nucleic acid fragment analysis system for pre-implantation genetic diagnosis.** *C. Akins*<sup>1</sup>, *S. Lilleberg*<sup>2</sup>, *M.C. Liu*<sup>3</sup>, *S. Kipersztok*<sup>3</sup>, *R.S. Williams*<sup>3</sup>, *K.C. Drury*<sup>3</sup>. 1) Transgenomic Inc., Houston, TX; 2) Applied Genomics and Molecular Genetics, Transgenomic Inc., Houston, TX; 3) Department of Obstetrics and Gynecology, University of Florida College of Medicine, Gainesville, FL.

Tri-nucleotide repeat expansion is the basis for a number of prevalent genetic disorders such as Huntingtons disease, Fragile X syndrome, and Myotonic Dystrophy. We investigated the use of single cell PCR to amplify a portion of the Huntingtons gene in order to develop a test system for pre-implantation genetic diagnosis (PGD). The amplification of CAG repeat sequences poses unique challenges due to the difficulty of denaturing high GC-content regions prior to PCR amplification. PCR modifications are necessary to carry out the amplification of GC-rich areas found in most tri-nucleotide expansions. We used a modified PCR protocol to efficiently amplify the expanded repeat sequence of the Huntington's gene. The amplified expanded CAG repeats were easily identified by denaturing high performance liquid chromatography (DHPLC) without the use of PCR primers labeled with fluorescent dyes. This technology shows great promise for sensitive and efficient detection of abnormal alleles in single cell samples.

**Limitations of preimplantation genetic diagnosis for a paternal carrier of a t(9;15) chromosome.** *V.A. Desilets<sup>1,2</sup>, S.L. Tan<sup>1</sup>, A. Ao<sup>1,2</sup>.* 1) OB/GYN, McGill University, Montreal, PQ, Canada; 2) Human Genetics, McGill University, Montreal, PQ, Canada.

**Introduction:** Preimplantation genetic diagnosis (PGD) is attractive to some parents who face a perceived high genetic risk. Its use is limited by the minute amount of material available.

**Case report:** This couple was referred to the McGill Prenatal Diagnosis Programme for preconception counseling regarding a paternal translocation (9;15) (p34;q11.2). They presented with primary male infertility for which intracytoplasmic sperm injection (ICSI) followed by in vitro fertilization (IVF) was planned. Our proband, aged 35 years, has severe oligospermia (CF negative) with unilateral varicocele. Blood karyotype revealed a translocation (9;15) (p24;q11.2). Subtelomeric FISH probe for 9p showed this segment to be translocated onto chromosome 15. His history was remarkable for a familial marker chromosome due to a 3:1 disjunction of t(9;15) in a grandparent, diagnosed prenatally with apparently normal outcome (published in 1989). His healthy wife was aged 35 years.

**Discussion:** The reproductive risks associated with the translocation included spontaneous abortion, unbalanced offspring and uniparental disomy (UPD). ICSI adds the risk of sex chromosome aneuploidy to that of autosomal aneuploidy due to maternal non-disjunction. As IVF/ICSI is planned as a treatment modality, PGD is available and may have possible benefit on implantation. Interphase fluorescence in situ hybridization (FISH) with specific probes for chromosome 9 and 15 can be performed on day 3 cleavage stage embryos to look for unbalanced chromosomal complement. Invasive PND is strongly recommended not only to confirm the results of preimplantation interphase FISH but for the following evaluations: subtelomeric probe for 9p deletion, UPD studies for 15q11.2, autosomal aneuploidy (maternal non-disjunction) or sex chromosome aneuploidy (ICSI).

**Conclusion:** Genetic counseling is an essential part of the PGD process to ensure informed consent from the parents.

**Predictive value of sperm chromosome analysis on the outcome of PGD for translocations.** *T. Escudero<sup>1</sup>, I. Addehadi<sup>2</sup>, M. Sandalinas<sup>1</sup>, S. Munn<sup>1</sup>.* 1) Preimplantation diagnosis, IRMS at Saint Barnabas, West Orange, NJ; 2) The Farah Hospital, Amman, Jordan.

Objective: To achieve a predictive relationship between the proportion of abnormal sperm and the proportion of abnormal embryos in couples with translocation male carriers. Setting: Private IVF center. Patients: 11 cases of reciprocal translocation male carriers. Intervention: Blood sample and sperm sample collection from each male partner. Embryo biopsy of the embryos produced in each cycle Main Outcome Measures: FISH on lymphocyte slides to characterize each translocation case. FISH with specific probes for each of the sperm sample with specific probes. Preimplantation genetic diagnosis of the translocations in the 11 cases. Results: Sperm results: A total of 11184 sperm were counted from the 11 patients included in the study. A total of 99 embryos from these 11 cases were biopsied, 93 giving results. Comparison between FISH Sperm Analysis and PGD Results indicated that there was no statistical difference between the segregation types observed in sperm and embryos. A correlation was found between the percentage of abnormal gametes and the percentage of abnormal embryos, and a predictive equation is proposed for this relationship:  $A = -55 + (1.9 \times B)$ , where A is the % of abnormal embryos and B the % of abnormal sperm. A total of 16 embryos were replaced in nine of the eleven cases. Four became pregnant, of which three are ongoing and one delivered a healthy normal baby. Conclusions: The predictive value of the sperm analysis is established with a proposed equation. Patients with 65% or less chromosomally abnormal sperm have a good chance at conceiving, while patients with higher rates would need to produce 10 or more good quality embryos to have reasonable chances at conceiving.

**FISH aneuploidy screen followed by translocation probe set on single blastomeres of embryos from translocation couple yield disappointing outcome.** *S.B. Olson<sup>1</sup>, H. Lawce<sup>1</sup>, N. Ouhibi<sup>2</sup>, D. Wolf<sup>2</sup>.* 1) Dept Mol & Med Genetics, Oregon Health & Sci Univ, Portland, OR; 2) Dept Obstetrics & Gynecology, Oregon Health & Sci Univ, Portland, OR.

With the application of FISH to the interphase blastomere as a screen for chromosomal imbalance, the opportunity has arisen for patients with balanced translocations to decrease the chance of miscarriage or an unbalanced child through embryo screening following IVF. Probe sets are designed, specific to the rearrangement, to potentially elucidate through a single hybridization, or through a hybridization, stripping and rehybridization sequence, all possible combinations of balanced and unbalanced segregation products. However, due to the high rate of aneuploidy in early embryos, further screening of the blastomeres for chromosomes such as 13,16,18,21,X and Y seems appropriate. A 31-year-old patient and her balanced 1p;8q translocation carrier husband produced 27 embryos. Twelve of these were frozen, leaving 15 for potential biopsy. Seven embryos progressed to day 3. Biopsy produced 6 blastomeres from 5 embryos appropriate for FISH. FISH for aneuploidy revealed one monosomy 21 male, one with only a single chromosome 13 signal, one haploid with an extra 13 (confirmed on a second blastomere), one uninformative and one balanced for the probe set. The balanced blastomere was rehybridized with the translocation probe set, which revealed 3 copies of the chromosome 1 short arm and 1 copy of the chromosome 8 long arm. What began as a promising venture with relatively young maternal age and a high yield of embryos resulted in no balanced embryos for transfer. Couples should be fully informed through extensive genetic counseling of the great possibility of disappointment.

**Polar Body Based Preimplantation Diagnosis for X-linked Disorders.** *Y. Verlinsky<sup>1</sup>, S. Rechitsky<sup>1</sup>, O. Verlinsky<sup>1</sup>, D. Kenigsberg<sup>2</sup>, J. Moshella<sup>2</sup>, V. Ivakhnenko<sup>1</sup>, C. Masciangelo<sup>1</sup>, C. Strom<sup>1</sup>, A. Kuliev<sup>1</sup>.* 1) Dept Molecular Genetics, Reproductive Genetics Inst, Chicago, IL; 2) Long Island IVF, Port Jefferson, NY.

Preimplantation diagnosis for X-linked disorders has been predominantly performed by gender determination, which, however, leads to discard of 50% unaffected male embryos. In an attempt to identify the X-linked mutation free embryos for transfer, we introduced preimplantation genetic diagnosis (PGD), using a sequential first and second polar body analysis, as an alternative to gender determination, which was offered to seven couples at risk for having children with X-linked genetic disorders, including hemophilia B, ornithine transcarbamylase deficiency, fragile- X syndrome, myotubular myotonic dystrophy and X-linked hydrocephalus.. The first and second polar bodies were removed following maturation and fertilization of oocytes in a standard IVF protocol and analyzed using a multiplex nested PCR, involving testing for mutations simultaneously with linked markers. Overall, 12 PGD cycles were performed, resulting in detection of 22 embryos with a predicted mutation free maternal contribution, which were transferred back to the patients in 12 cycles, yielding 4 clinical pregnancies, three already resulting in birth and one currently ongoing. The results suggest the clinical usefulness of polar body testing for X-linked disorders as an alternative to PGD by gender determination.

**Detection of expanded CAG repeats at the myotonic dystrophy (DM) locus in single cells by triplet primed PCR (TP-PCR) for preimplantation genetic diagnosis (PGD).** *P.F. Ray<sup>1</sup>, N. Frydman<sup>2</sup>, R. Frydman<sup>2</sup>, M. Vekemans<sup>1</sup>, A. Munnich<sup>1</sup>.* 1) Departement de genetique medicale, Hopital Necker, PARIS; 2) Service de genicologie-obstetrique, Hopital Antoine Beclere, Clamart, France.

The amplification of large number of triplet repeats is challenging from microgram amounts of DNA but is impossible from single cells. Large pathologic alleles responsible for myotonic dystrophy (DM) can expand up to several thousands triplets but a maximum of 50-100 repeats can reliably be amplified from single cells. Thus, genetic analysis in the course PGD for couples at risk of transmitting myotonic dystrophy has so far mostly been based on the detection of the normal allele of the transmitting parent. This semi-indirect strategy, however, is only applicable when that allele is different in size from the propositus partner two alleles, from our experience, only about one fourth of the couples. Warner et al. (1996)\* described a fluorescent assay in which a primer internal to the triplet repeat itself was used and allowed the detection of expanded alleles. We utilized this strategy and developed a single cell hemi nested TP-PCR assay. The outer reaction mix contained a DM specific forward primer and the TP primer made of 7 CAG repeats tailed with an overhanging 20 bp specific primer. Aliquots of this reaction were reamplified with the same 5 fluorescently labeled primer and the 20 nucleotides specific primer. Fluorescent TP-PCR products were analyzed in an ABI 310 DNA analyzer. Expanded alleles were detected in 100% of the control lymphocytes analyzed and one PGD cycle was carried out. Following this procedure two embryos which had not shown amplification of the expanded allele were transferred. Positive hCG were detected two weeks later, indicating a successful uterine implantation of an embryo but the pregnancy was not sustained. Reliable detection of expanded CAG repeats was achieved from single lymphocytes and blastomeres by fluorescent TP-PCR. This single cell analysis technique is applicable to all couples at risk of transmitting myotonic dystrophy and similar protocols could be developed for the diagnosis of other trinucleotide expansion diseases. \* J Med Genet, 1996, (33) 1022-6.



**Clinical Assessment of Routine Chromosome Analysis and Rapid Interphase Fluorescence in situ Hybridization (FISH) in Prenatal Diagnosis: Evaluation of 993 New Cases.** *S.A. Ebrahim<sup>1</sup>, M.I. Evans<sup>2</sup>, A. Johnson<sup>1</sup>, M.P.*

*Johnson<sup>3</sup>, A.A. Al-Katib<sup>1</sup>, A.N. Mohamed<sup>1</sup>.* 1) Dept Pathology, Cytogenetics, Ob/Gyn, Wayne State Univ, Detroit, MI; 2) Dept Ob/Gyn&Human Genetics MCP Hahnemann Univ, PA; 3) Children's Hospital of Philadelphia, PA.

Over a 5-year period, we have carried out 1294 FISH studies on uncultured chorionic villous samplings, amniotic fluid, or fetal blood from women at high risk for having a child with a chromosomal anomaly. The results of the first 301 samples have been previously described (*Am J Med Genet* 2000;90:233-8). We presently report on an additional 993 new cases. FISH analyses have been routinely performed in our cytogenetics laboratory on uncultured fetal cells using the commercially available probes specific for chromosomes 13, 18, 21, X, and Y. Among the 5009 prenatal samples analyzed between July 1998 and May 2001, both FISH and conventional chromosome studies were performed on 993 (19.8%) cases. Aneuploidies were detected by FISH in 72 (7.3%) samples. Of those, 40% were trisomy 21, 28% trisomy 18, 9.7% trisomy 13, 9.7% monosomy X, 1.3% XXX, 1.3% XXY, 1.3% monosomy 21\*, and 8% were triploidy. All FISH results were reported in 8-48 hr. Subsequently, all FISH results were confirmed by chromosome analysis. In 18 (1.8%) cases with normal FISH results, chromosome analysis revealed abnormalities that could not be detected by FISH. However, in two cases with ultrasound anomalies, there was a discrepancy between FISH and the initial chromosome analysis. The first case was found to be trisomy 21 by interphase FISH, however, chromosome analysis revealed only a diploid 46,XY karyotype. FISH on metaphase cells revealed the presence of a cryptic unbalanced translocation: 46,XY,der(6)t(6;21)(q27;q22.2). The second case was determined to be monosomic for chromosome 21\* by interphase FISH. Subsequent chromosome and FISH analyses revealed a 46,XX, idic(21)(q22).ish idic(21)(q22.12)(D21S55-). Our data demonstrates the clinical utility and the accuracy of interphase FISH for rapid prenatal identification of specific chromosome aneuploidies, including rare chromosome rearrangements that likely would have been missed in the absence of FISH.

**First trimester maternal plasma detection of fetal specific DNA: Anti-coagulant influences sensitivity of real-time PCR.** *F.Z. Bischoff<sup>1</sup>, D.X. Dang<sup>1</sup>, D.A. Marquez-Do<sup>1</sup>, D. Lewis<sup>2</sup>, C. Horne<sup>2</sup>, A. Burke<sup>1</sup>, J.L. Simpson<sup>1,3</sup>.* 1) Depts of OB/GYN; 2) Immunology; 3) Molecular/Human Genetics, Baylor College of Medicine, Houston, TX.

Fetal DNA is present in maternal plasma and may be used for noninvasive prenatal genetic diagnosis. Although highly sensitive during the 2nd and 3rd trimesters, genetic testing during the 1st trimester has been considered less efficient due to relatively lower concentrations of fetal DNA. Because 1st trimester testing is preferable for early diagnosis and pregnancy management, determining optimal conditions for processing plasma DNA are necessary. **OBJECTIVE:** To determine whether anti-coagulant influences the detection of fetal sequences in 1st trimester samples. **METHODS:** Peripheral blood was obtained from women prior to CVS or amniocentesis at 10.9-21.7 weeks gestation (mean 15.9 wks). Blood was collected in vacutainers containing ACD (n=10), EDTA (n=5) or NaHep (n=11). In cases confirmed with a male fetus (n=28), plasma DNA was purified (Qiagen). Real-time PCR was performed using the TaqMan assay to detect the FCY (Yq11.2) and GAPDH (control) locus. Fetal DNA concentration (copies/ml plasma) was determined using the Sequence Detection System software (PE Applied Biosystems). **RESULTS:** See Table. **CONCLUSIONS:** EDTA improves efficiency of fetal sequence detection in 1st trimester cases. Combined with Real-time PCR, this approach may prove to be valuable for noninvasive prenatal detection of paternally derived sequences.

Anit-Coagulant	First Trimester	Second Trimester	Total
EDTA	4/4 (100%)	1/1 (100%)	5/5 (100%)
ACD	1/3 (33%)	4/7 (57%)	5/10 (50%)
NaHep	1/3 (33%)	5/8 (63%)	6/11 (55%)

**Personal preference assessments of prenatal diagnosis.** *W.A. Grobman, S.L. Dooley, E. Welshman, E. Calhoun, E. Pergament.* Northwestern University Medical School, Chicago, IL.

**Purpose:** The formulation that women should be offered invasive prenatal testing when the risk of having a child with Down syndrome exceeds the risk of having a procedure-related miscarriage is potentially specious. The underlying rationale is that women perceive the birth of a child with Down syndrome as a health state equal to that of a miscarriage. This rationale, developed without examining actual patient preferences, remains an unproven assumption. The objective of this study was to determine if patients' utility values (quantitative assessments of patients' perceptions of health states) support maternal age  $\geq 35$  years for the offering of invasive prenatal testing. **Method:** An interview-administered survey was conducted with 187 pregnant women receiving antepartum care at a university hospital to determine demographic characteristics, attitudes toward prenatal diagnosis, and utilities for spontaneous abortion (SAB) and the birth of a child with Down syndrome (DS) of varying clinical severity. Utility values were determined through the use of a standard gamble paradigm. Lower utility values reflect less desirable health states. **Results:** SAB of a pregnancy had a mean utility of .76. The utility for the birth of a child with DS decreased ( $p < .001$ ) as the clinical severity of DS increased from mild (.78) to severe (.65). The only factor that predicted a difference between the utility for birth of a child with Down syndrome and miscarriage was the desire for amniocentesis. Women who desired amniocentesis had a utility value for SAB (.79) that was significantly higher ( $p < .01$ ) than for the birth of a child with DS of unknown severity (.73), while women who did not desire amniocentesis did not show a difference in the utility values. **Conclusions:** Women who desire prenatal genetic testing do not perceive the birth of a child with DS and a miscarriage to be equivalent health states. The appropriateness of the 35-year-cut-off is debatable. Patient preferences may neither be congruent with economic interests of the health care system nor correspond to traditional risk vs benefit concepts based on maternal age.

**Non-invasive prenatal diagnosis of congenital adrenal hyperplasia through the use of fetal DNA in maternal plasma.** R.W.K. Chiu<sup>1</sup>, T.K. Lau<sup>2</sup>, Z.Q. Gong<sup>3</sup>, P.T. Cheung<sup>3</sup>, T.N. Leung<sup>2</sup>, Y.M.D. Lo<sup>1</sup>. 1) Department of Chemical Pathology; 2) Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong; 3) Department of Paediatrics, The University of Hong Kong, Hong Kong SAR, China.

The presence of fetal DNA in maternal plasma has allowed the development of strategies for non-invasive prenatal diagnosis. However, as fetal DNA circulates in maternal plasma among a high background of maternal DNA, applications reported to date have been limited to the prenatal investigation of autosomal dominant, paternally-inherited genetic traits, such as rhesus D, myotonic dystrophy and achondroplasia. In this study, we investigated if maternal plasma analysis could be used for the prenatal diagnosis of autosomal recessive (AR) disorders, using congenital adrenal hyperplasia (CAH) as a model system. For AR conditions, the ability to detect a paternally-inherited mutation in maternal plasma does not aid in disease diagnosis, as it does not clarify whether the fetus is a homozygote or heterozygote. Consequently, we have taken the approach to detect the presence of the *normal* paternal allele through the use of polymorphic markers, whose presence in maternal plasma effectively excludes the diagnosis of the AR condition. A couple, whose first child was diagnosed with 21-hydroxylase (encoded by *CYP21*) deficiency, presented during their second pregnancy for prenatal diagnosis. Both intragenic and extragenic (HLA-haplotype) markers have been used to characterize the normal paternal allele. The family was found to be informative for 3 polymorphic sites in intron 2 of *CYP21*. Primers were designed to amplify the polymorphic sites, as well as HLA class II genes. Both the intragenic polymorphic markers and the HLA class II gene associated with the normal paternal allele were amplifiable in maternal plasma collected at 17 weeks of gestation, which supports the conclusion that the unborn baby is not a compound heterozygote for 21-hydroxylase deficiency. The results have been confirmed by HLA-typing and mutation analysis of amniotic fluid. The use of fetal DNA in maternal plasma for the non-invasive prenatal diagnosis of CAH is possible, and this approach could potentially be applied to other AR conditions.

**Genetic management of adverse outcomes in a semen bank.** *R.N. Dudovitz<sup>1</sup>, M. Ray<sup>2</sup>, R.E. Falk<sup>1</sup>.* 1) Medical Genetics-Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, CA; 2) California Cryobank, Los Angeles, CA.

A total of 355 adverse reaction reports (ARRs) from artificial insemination with donor sperm, collected by a single semen bank over a 14 year period, were examined to investigate the genetic management and implications of such cases. Data was reviewed by a single geneticist and classified regarding the likelihood of chromosomal, multifactorial, Mendelian, or other causation. Eighty-six ARRS reported chromosomal abnormalities, 172 were non-chromosomal, 56 described maternal complications (infection, fever), and 41 noted an unexplained fetal demise. Excluding multifactorial traits, 18 of 299 (6%) non-maternal ARRs were clearly donor related. Five of the 18 donor-related ARRs were chromosomal and could be avoided by cytogenetic analysis of potential donors. The semen bank was dependent on the client or her physicians for access to medical records and laboratory reports, limiting the accuracy of genetic risk assessment. Nearly 22% of ARRs did not identify the client or allow direct discussion with her. While 55% of ARRs were reported by the client, many allowed no contact with or reporting from the child's physicians. Overall, 70% of diagnoses remained vague or undocumented despite repeated attempts to obtain additional information. The interval between birth of the child and notification of the semen bank varied considerably as 66.7% of ARRs were reported within 1 year, 18% within 2-4 years and 9.5% by 4-9 years. In some cases this reflected late manifestation of the condition, while other cases were reported after considerable delay. As repeated use of a particular donor could lead to recurrence of a genetic condition, timely and accurate reporting of even potentially genetic adverse outcomes is critical. Although there are guidelines for screening semen donors, there are no regulations regarding donor screening or follow-up of reported abnormal outcomes at present. Continued use of a specific donor's semen after report of an adverse outcome raises a number of social and ethical issues which will be reviewed. These may influence policy decisions regarding regulation of donor screening and follow-up of adverse outcome reports in semen banking.

**Novel approaches for the prevention of Types A and B Niemann-Pick Disease: Enrichment of normal from mutant sperm in heterozygous mice.** *A. Butler, H.W. Snoeck, K.A. Kelley, R.E. Gordon, E.H. Schuchman.* Dept Human Genetics, Mount Sinai School of Medicine, New York, NY.

Types A and B Niemann-Pick disease (NPD) are lysosomal storage disorders resulting from the deficient activity of acid sphingomyelinase (ASM) and the subsequent accumulation of sphingomyelin and cholesterol. The carrier frequency for Type A NPD among Ashkenazi Jewish individuals is ~ 1:80, with a predicted 25% risk of an affected fetus being born to carrier couples. No treatment is currently available for this disorder. The goal of our research was to develop a pre-fertilization, gamete selection technique that could significantly reduce or eliminate the chance of producing an affected fetus from NPD carrier couples. Our laboratory previously constructed an ASM knockout mouse model of NPD and data obtained from *in vivo* mating studies suggested that NPD mice exhibited reduced fecundity. Investigation of male reproductive physiology by light and electron microscopy revealed accumulation of lipid filled vacuoles within Sertoli cells of the testes and epithelial cells of the epididymis of affected mice. Mature sperm from affected mice also showed various functional and physical abnormalities, including accumulation of lipid, reduced motility, "hairpin" structures, and increased cell death. While sperm from normal mice did not exhibit any of these findings, those from heterozygous mice exhibited several features that were intermediate between normal and affected mice. Consequently, we present data on the enrichment of normal sperm from heterozygous mice by (I) flow cytometry based on mitochondrial membrane potential, acrosome status, or general viability, and (II) physical separation techniques based on motility or density. These results suggest that adult male NPD patients and carriers may also have sperm abnormalities that lead to reduced fertility, and that ASM is essential for normal sperm development.

**Absent eyelids, micrognathia, digital and skeletal abnormalities and ambiguous genitalia: A new autosomal recessive syndrome.** *D. Chitayat*<sup>1,2,3</sup>, *C. Sherman*<sup>1,3</sup>, *S. Viero*<sup>1,3</sup>, *D. Myles-Reid*<sup>1,3</sup>, *A. Toi*<sup>1,3</sup>. 1) Mount Sinai Hospital; 2) Hospital for Sick Children; 3) University of Toronto, Toronto, ON, Canada.

Absent eyelids is a rare finding. We report male and female fetuses born to a consanguineous Pakistani couple with absent eyelids, skeletal abnormalities and absent external genitalia. The couple's first pregnancy was complicated with ultrasound findings of oligohydramnios, dolichocephalic skull, cardiac abnormalities, kyphoscoliosis and splaying of the lamina in the lower lumbar spine, at 19 weeks. The pregnancy was terminated and the autopsy showed a globular and hyperextended head, with prominent occiput, absent eyelids, small pointed nose, micrognathia, nuchal edema, webbed neck, absent clavicles, right axillary pterygia, hypoplastic thumbs with tapering fingers, partial syndactyly of the 3<sup>rd</sup> and 4<sup>th</sup> fingers, limitation in extension of both knees with popliteal pterygia, bilateral clubbed feet, syndactyly between the 4<sup>th</sup> and 5<sup>th</sup> toes, imperforated anus and prominent clitoris with labia or vaginal opening. Internal examination showed Ebstein cardiac anomaly, moderate hypoplasia of the left ventricle, hypoplastic lungs and unilobar left lung, and female internal genitalia. The karyotype was 46,XX. The couple's second pregnancy was a few months later. A fetal ultrasound at 20.1 weeks revealed fetal measurements consistent with 17.2 weeks gestation and multiple abnormalities including oligohydramnios, hypertelorism, dolichocephaly, lateral cerebral ventriculomegaly, hyperextended neck, flexed arms, lordotic thoracic spine, kyphotic lumbar spine, thin ribs, query absent clavicles, prominent right eye and a pterygium over the left elbow. The couple decided to terminate the pregnancy and autopsy was not consented. External examination showed the same manifestations as in the previous fetus. The karyotype was 46,XX. Although the findings have some features in common with Neu-Laxova syndrome, the absence of brain abnormalities, the abnormal genitalia, absent clavicles and normal skin differ between these conditions. This is thus a hitherto new autosomal recessive disorder.

**The Fragile X Syndrome: Screening and prenatal diagnosis.** *W.T. Brown, S. Nolin, G.E. Houck, X.H. Ding, A. Glicksman, S.H. Li, S. Brooks, C. Dobkin, E.C. Jenkins.* Dept Human Genetics, NYS Inst Basic Research, Staten Island, NY.

Fragile X syndrome is the most common inherited form of mental retardation. Its molecular basis is usually an expansion of a repetitive CGG triplet sequence located in the 5' untranslated region of the fragile X gene, FMR1. Because of its prevalence and medical importance, efficient means for accurate diagnostic screening and prenatal testing are needed. Polymerase chain reaction (PCR) is rapid and requires little DNA. We have carried out screening and prenatal diagnosis for the fragile X syndrome using a PCR protocol we have developed that accurately resolves normal alleles as well as detects premutations and most full mutations (>90%). Follow-up Southern blotting is carried out on prenatal samples and other selected samples. Testing of 2095 males with developmental delay of unknown etiology revealed 61 (3%) were positive for fragile X. Our prenatal experience now includes screening of 624 pregnant women, who had a family history of mental retardation of undetermined etiology. We have found 12 of the women to be carriers: 3 full mutations, 3 premutations and 6 with unstable borderline alleles. Two additional fragile X families were identified although the women were not carriers. Thus, 2.7% (14/624) of women were carriers or came from fragile X families or had unstable alleles. Additionally, 25 carriers (40%) with prior unknown carrier status were identified among 62 pregnant members of previously identified fragile X families. Prospective prenatal testing of 206 carrier women correctly detected 90 fetal samples with full mutations and 15 with premutations. Follow up information on all samples obtained so far, including approximately 30% of the terminated products of conception, indicates no false positive or negative results. Maternal cell contamination has presented problems in 3 cases. Thus, highly reliable screening of at-risk pregnant women for fragile X status and subsequent prenatal diagnosis is now possible.



**Genetic Counseling and Prenatal Diagnosis of X-Linked Hydrocephalus and Tetralogy of Fallot.** *M.T. Jodah<sup>1</sup>, B.G. Kousseff<sup>1</sup>, J.L. Angel<sup>2</sup>*. 1) Dept. of Pediatrics, USF Regional Genetics Program, Tampa, FL; 2) Florida Perinatal Associates, Tampa, FL.

Congenital hydrocephalus occurs in approximately 0.4-0.8/1,000 livebirths and stillbirths. It may be caused by chromosome abnormalities, neural tube defects, infections, teratogens, trauma, syndromic and non-syndromic conditions and mendelian disorders. X-linked hydrocephalus with aqueductal stenosis (HSAS; MIM #307000) is the most common genetic form of congenital hydrocephalus; incidence is estimated at 1/30,000. The phenotype shows mental retardation, macrocephaly and adducted thumbs; aqueductal stenosis may or may not be present. Approximately 5% of cases with hydrocephalus belongs to this type. The gene, L1CAM, (L1 cell adhesion molecule), was mapped to Xq28, along with three other genetic conditions: MASA (mental retardation, aphasia, shuffling gait), ACC (agenesis of the corpus callosum), and SPG1 (spastic paraplegia type 1). This represents allelic heterogeneity with mutations in the same gene resulting in different phenotypes. In HSAS, associated anomalies typically involve the central nervous system. To our knowledge, this is the first reported case involving prenatal diagnosis of X-linked hydrocephalus with Tetralogy of Fallot.

**Fetal diaphragmatic hernia and upper limb anomalies suggest Brachmann-de Lange syndrome.** *T. Marino<sup>1</sup>, P.G. Wheeler<sup>2</sup>, L.L. Simpson<sup>3</sup>, S.D. Craigo<sup>1</sup>, D.W. Bianchi<sup>2</sup>.* 1) Division of Maternal-Fetal Medicine, Obstetrics & Gynecology;; 2) Division of Genetics, Pediatrics; New England Med Ctr, Boston, MA; 3) Obstetrics & Gynecology, Columbia-Presbyterian Med Ctr, NY, NY.

With the routine use of ultrasound imaging for fetal structural anomalies, diaphragmatic hernia is now increasingly diagnosed prenatally. In the absence of associated anomalies, or when associated anomalies are not identified before birth, prospective parents are initially referred to a pediatric surgeon to discuss postnatal surgical options. The subsequent detection of associated fetal anomalies may create difficulties for a family that has been counseled with an emphasis on surgical repair, as opposed to a syndrome diagnosis.

We describe two independent cases of Brachmann-de Lange syndrome in which second trimester fetal ultrasound evaluation showed the presence of a diaphragmatic hernia and upper limb anomalies. In both cases the fetal karyotype was normal and the prospective parents were referred for pediatric surgical consultation. Intrauterine growth restriction developed in the third trimester. Postnatal and post-mortem physical examinations demonstrated typical physical findings associated with Brachmann-de Lange syndrome. The prenatal diagnosis of diaphragmatic hernia should prompt an intensive search for additional anomalies. If upper limb anomalies are detected an underlying diagnosis of Brachmann-de Lange syndrome should be considered.

**Investigation of prenatal samples with ultrasound findings suggesting dwarfing syndromes.** *A.Q. Hejmanowski<sup>1</sup>, A. Sommer<sup>2</sup>, T.W. Prior<sup>1</sup>*. 1) Pathology, Ohio State University, Columbus, OH; 2) Children's Hospital, Columbus, OH.

Thanatophoric Dysplasia (TD) is the most common form of lethal dwarfism. 48.4% (46 of 95) of the Molecular Pathology laboratory's prenatal samples are for a skeletal dysplasia panel, which includes achondroplasia (ACH), hypochondroplasia (HCH), and TD. 68.2% (28 of 41) of the TD samples mention abnormalities on ultrasound, especially micromelic limb shortening, macrocephaly with frontal bossing, and flattened vertebrae, as the reason for submission. Our lab tests for the two most common mutations causing TD: Lys650Glu, which is the only mutation known to cause TD type II, and Arg248Cys, the mutation most often found in TD type I. 86.9% (40 of 46) of our samples are negative for these mutations, as well as mutations that cause ACH or HCH. The present study was conducted to screen the negative patients for other mutations in the Fibroblast Growth Factor Receptor 3 (FGFR3) gene.

These samples were sequenced using PCR and the Applied Biosystems BigDye Terminator cycle sequencing kit. The FGFR3 coding sequence contains 19 exons. Exons 2 through 18 have been sequenced in 11 patients. Two (18.2%) were found to have the second most common TD I causing mutation, Ser249Cys. The other nine (81.8%) were found to have no mutations. Two other patients have been partially sequenced (three exons remain to be finished) with no findings. A test for the Ser249Cys mutation should be added to skeletal dysplasia screen, and is in development in the Molecular Pathology lab. In samples dating from March of 1997 through the present, we have found only five TD mutations following abnormal ultrasounds. This strongly supports the conclusion that ultrasound, though a powerful tool for diagnosis of some form of abnormality, is not specific enough to be used for a differential diagnosis of a specific dwarfing syndrome. Secondly, it shows that other genes may cause ultrasound findings that mimic TD.

**Sensitivity of fluorescence in situ hybridization to correctly identify chromosomal abnormalities in high risk obstetrical populations.** *K. Mujaibel<sup>1</sup>, H. Bruyere<sup>2</sup>, S. Langlois<sup>3</sup>, R.D. Wilson<sup>1,3</sup>.* 1) Obstetrics and Gynecology; 2) Pathology; 3) Medical Genetics, University of British Columbia, Canada.

**STUDY DESIGN:** A retrospective chart review of all patients seen between 1998-2000 who underwent a genetic amniocentesis and were offered FISH on uncultured amniocytes as an adjunct to conventional chromosomal analysis because of a gestational age >21 weeks or an ultrasound finding of a fetal anomaly highly suggestive of aneuploidy. The study population was divided into two groups: group I included cases with U/S finding of fetal anomalies and/or abnormal fetal growth and/or abnormal fluid volume; group II included cases with U/S findings of markers of aneuploidy, some of which also had positive triple screen or AMA. FISH results were then compared to the conventional chromosomal analysis. **RESULTS:** A total of 150 patients charts were evaluated. Mean maternal age at amniocentesis was 29.9 years. Mean gestational age at amniocentesis was 20 weeks. The rate of chromosomal abnormality in the total study population was 27% (40/150) as detected by the conventional karyotype. FISH identified 36/40 of the chromosomal abnormality with an overall sensitivity of 90%. In group I, FISH accurately identified the chromosomal abnormality in 92% (22/24) of positive cases. In group II, FISH was positive in 88% of the chromosomal abnormality cases (14/16). When evaluated by the type of the US abnormality, FISH was 100% accurate in identifying abnormal chromosomes in the group with CNS and GI abnormalities and 71% (5/7) sensitive in cases of abnormal CVS. In the presence of multiple major US findings, the sensitivity of FISH was 88% (7/8) and 83% (5/6) in cases of multiple markers of aneuploidy. There were 4 false negative results with FISH (4/40). Three were structural chromosomal abnormalities that would not be expected to be identified with the use of FISH (2 translocations and one mosaicism). The fourth result was reported as inconclusive and the final karyotype was trisomy 18. **CONCLUSION:** In a population at high risk for aneuploidy, FISH was a clinically useful rapid prenatal diagnostic test with a sensitivity of 90% and NPV of 96% in detecting significant chromosomal abnormalities.

**Severe Knee Hyperextension In A Fetus With Trisomy 21: Association or Coincidence?** *M.J. Huggins<sup>1</sup>, P.T. Mohide<sup>1</sup>, J. Bourgeois<sup>3</sup>, M.J.M. Nowaczyk<sup>2,3</sup>*. 1) Obstetrics and Gynecology; 2) Pediatrics; 3) Pathology and Molecular Medicine, Hamilton Health Sciences and McMaster University.

**CASE REPORT:** A 28 year old woman in her 2nd pregnancy was referred after abnormal findings on a screening 18 week ultrasound. Detailed sonographic evaluation revealed a singleton fetus with hyperextended knees and feet. Fetal echocardiography confirmed the presence of tetralogy of Fallot with a large perimembranous/outlet ventricular septal defect and aortic override. The nuchal fold measured 6.4 mm at 20 weeks of gestation. Interphase FISH of amniocytes showed that 90% of cells had an additional hybridization signal indicative of trisomy 21. Following genetic counselling, the couple requested pregnancy termination. **RESULTS:** Postmortem examination of the fetus revealed typical facial features of trisomy 21, with low set ears, short palpebral fissures, broad face and short nasal bridge. There were bilateral single palmar creases and clinodactyly. The cardiac findings were tetralogy of Fallot with overriding aorta, VSD, and small left ventricular outflow tract. There was hyperextension of the knees bilaterally, with possible hypoplasia of the anterior cruciate ligaments. **DISCUSSION:** The association between Down syndrome (DS) and joint hyperflexibility is well documented, but mainly confined to the cervical region and the hip joints. The severe hyperextension of both the knees seen in this case has not been previously reported as a prenatal feature of DS, although in view of other joint abnormalities in DS, this unusual finding may be consistent with that diagnosis. This case would suggest that a sonographic finding of hyperextended knees should be considered a possible indication for fetal karyotyping.

**Pitfalls in prenatal diagnosis of the 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) due to complicated gene conversion and rearrangements.** *R. Mao<sup>1</sup>, L. Nelson<sup>1</sup>, R. Kates<sup>2</sup>, C. Miller<sup>3</sup>, W. Tang<sup>1</sup>, K. Ward<sup>1</sup>.*  
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Steroid 21-hydroxylase deficiency is the cause approximately 90% of the CAH patients. Although biochemical testing remains the primary method of postnatal diagnosis, DNA analysis has assumed a central role in prenatal assessment of at-risk pregnancies due to its accuracy in mutation identification. Pitfalls to prenatal diagnosis can be related to the presence of the highly homologous 21-hydroxylase pseudogene, CYP21P, adjacent to the functional gene, CYP21. We report two cases of complex gene conversion and rearrangement between the CYP21 and CYP21P posing unique prenatal diagnostic challenges. **Patients:** In Family 1, a 5 y/o girl was diagnosed in an early infancy with CAH several weeks after presenting with dehydration and salt wasting. She had an enlarged clitoris and elevated 17-hydroxy progesterone level. The 18-month-old boy in Family 2 was identified by an elevated 17-hydroxy progesterone level upon newborn screening. Both of the families requested prenatal testing for the current pregnancies. **Methods:** Analysis of eight common mutations and the deletion in the CYP21 gene is accomplished using PCR followed by specific enzyme digestion and Southern Blot. Linkage analysis was performed using the microsatellite markers flanking the CYP21 gene. **Results:** Results from the mutation detection indicated a complicated gene conversion and rearrangement in the affected individual in both of the families. Interpretation of these changes made it difficult to determine whether the fetus(es) in the current pregnancies would be affected with CAH. Linkage study revealed that each fetus had inherited both mutated alleles from the parents and was therefore predicted to be affected with CAH. **Conclusion:** As observed in the two reported cases, gene conversion or rearrangement between the CYP21 and CYP21P can provide limitations to the scope of information yielded from DNA. We suggest that supporting the direct mutation detection with linkage study will provide more comprehensive information for the family.

**Experience in the prenatal diagnosis of the fetus with holoprosencephaly.** *D. Mayen<sup>1</sup>, S. Garza<sup>2</sup>, R. Garcia-Cavazos<sup>1</sup>, T. Leis<sup>1</sup>, M.J. Zavaleta<sup>1</sup>.* 1) Dept Genetics, Inst Nacional de Perinatologia, Mexico City, Mexico; 2) Dept Neurology, Hosp Infantil de Mexico "Federico Gomez, Mexico City.

**Introduction:** Holoprosencephaly (HPE) is a spectrum of cerebral abnormalities resulting from incomplete cleavage of the forebrain. Up to 45% have chromosomal abnormalities, 25% monogenic syndromes and other aetiologies (teratogenic agents diabetes mellitus). **Purpose:** Describe the perinatal management of the fetus with HPE in a mexican population. **Methods:** All pregnant women with a fetus with hydrocephaly and /or medium-line craneofacial anomalies detected with conventional ultrasound during the period between june 1993 and april 1999 were included. We studied family history and performed physical examination of both parents and high resolution ultrasound with videofilmation, amniocentesis for chromosomal analysis and fluorescence *in situ* hybridization. At birth we comfirmed the diagnosis by physical examination, transfontanelar ultrasound, CT/MRI or autopsy. **Results:** We detected 120 cases with hydrocephaly and/or medium-line craneofacial malformations, of which 20 cases(16.6) were HPE(16 alobar and 4 semilobar): The mean gestational age at the comfirmation of the diagnosis were 27.4 weeks(range 15 to 36.5). Five cases(0.25) were associated with maternal diabetes, six(0.30) had chromosomal anomalies: 5 were associated with the critical points for HPE(two with partial monosomy 18p and cyclopia). **Conclusion:** HPE is a common cause of fetal hydrocephaly and the correct diagnosis and perinatal management required a multidisciplinary group.

**Cultivation of fetal erythroid precursors out of maternal blood: Their isolation and characterisation with PCR and FISH.** *H. Hohmann<sup>1</sup>, S. Michel<sup>1</sup>, W. Reiber<sup>2</sup>, C. Kaehler<sup>3</sup>, U. Claussen<sup>1</sup>, F. von Eggeling<sup>1</sup>.* 1) Inst Human Genetics, Univ Jena, Jena, Germany; 2) Private Gynecologist, Univ Jena, Germany; 3) Institute of Gynecology, Univ Jena, Germany.

The cultivation of fetal cells out of maternal blood may be an alternative in non-invasive prenatal diagnostic. The percentage of fetal cells could be improved in vitro by increasing their number with a selective culture medium. In our experiments 10 ml blood was used from 22 women carrying a male fetus. We used triple density gradient centrifugation to isolate mononuclear cells and the fraction containing most erythroblasts. The pooled cells were cultivated 10 to 14 days in haematopoietic growth medium. Red (CFU-E, BFU-E) and white coloured cell clones were individually collected by micromanipulation. A part of each clone was characterised by Y-specific PCR-systems, using the Amelogenin- and the SRY-detection System. If the PCR result was Y-positive the remaining cells of the clone were used to carry out fluorescence in situ hybridisation (FISH) to detect XY-positive interphase nuclei and metaphases. PCR results showed that with SRY PCR 12.3 % (n = 40/323) of the collected red clones tested were of fetal origin and 6.1 % (n = 32/522) of the clones analysed by Amelogenin PCR were Y-positive. In contrast all collected white cell clones were Y-negative. FISH analyses of PCR-positive clones revealed that within one individual clone only up to 30% of the cells are of fetal origin. No Y-PCR positive red clone showed exclusively fetal cells, which might be a kind of contamination or more possible it could be characteristic for clonal fetal cell growth as they may require maternal cells for stimulation. Our results show that the cultivation and preparation of fetal progenitor cells is possible but still need to be improved for reliable prenatal diagnosis.



**Molecular characterization of  $\alpha$ -thalassemia genes in an Iranian population.** *M.A. Kerachian<sup>1</sup>, M. Houshmand<sup>2</sup>, A. Bani-Hashem<sup>3</sup>, M. Gholamin<sup>1</sup>, R. Vakili<sup>1</sup>, M.R. Abbaszadegan<sup>1</sup>.* 1) Dept. of Human Genetics, Bu-Ali Res Ins, Mashad Univ. Med. Sci., Mashad, Iran; 2) NRCGEB, Tehran, Iran; 3) Sheik Children's Hospital, Mashad, Iran.

Alpha-thalassemia is the most common inherited disorder of hemoglobin (Hb) synthesis in the world. More than 95% of recognized  $\alpha$ -thalassemia involves deletion of one or both  $\alpha$ -globin genes from chromosome 16p13.3. Determination of the underlying genotype is especially important for reproductive planning because parents with two gene *cis* deletions ( $\alpha/--$  or  $- \alpha/--$ ) can have offspring with the fetal condition hemoglobin (Hb) Bart's hydrops fetalis ( $--/--$ ) or Hb H disease ( $- \alpha/--$ ). We used polymerase chain reaction (PCR) to identify couples at risk of conceiving fetuses afflicted with Hb H disease or Hb Bart's hydrops fetalis syndrome. Furthermore, specimens of suspected patients for  $\alpha$ -thalassemia were studied. This study sought to detect three different  $\alpha$ -thalassemia deletions ( $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$  and  $--SEA$ ) among a population in northeastern Iran, Mashad. Our study indicates that more than one of two couples experience  $\alpha$ -thalassemia. However, about 5% are  $\alpha$ -thalassemia trait. Amongst patients ( $n=46$ ), there were no  $\alpha$ -thalassemia with  $-\alpha^{4.2}$  and  $--SEA$  deletions. Sequencing the amplified fragments verified our results. Therefore, the PCR approach may have major implications for population screening at prenatal diagnosis programs aimed at secondary prevention of  $\alpha$ -thalassemias.

**Prospective Study of Molecular Fragile X Syndrome Prenatal Diagnosis.** *J. Mallolas<sup>1</sup>, C. Badenas<sup>1</sup>, M. Rife<sup>1</sup>, A. Soler<sup>1</sup>, A. Borrell<sup>2</sup>, A. Sanchez<sup>1</sup>, M. Mila<sup>1</sup>.* 1) Genetics, Hospital Clinic, Barcelona, Barcelona, Spain; 2) Unidad de Diagnostico Prenatal Hospital Clinic. Barcelona.

Since 1992, sixty-five prenatal diagnoses for fragile X syndrome have been performed. Among them, 51 correspond to fragile X syndrome pedigrees (41 belonging to a carrier mother and 10 to at risk mothers). The remaining 14 corresponded to mothers with no expansion in the FMR1 gene, where the molecular study was simultaneously done in the mother and fetus due to a family story of mental retardation. A parallel cytogenetic and molecular study was performed in all cases. Molecular study consisted in both PCR and Southern blot (StB12.3 probe after double digestion with EagI and EcoRI). When necessary, indirect study was done using AC1, AC2 DXS548 and DXS297. We were able to reach a diagnosis in all cases but one (98.4%). In 7(10.7%) cases a second sample was obtained due to abnormal methylation patterns. Regarding the 41 pregnancies from carrier mothers, 20 were normal (10 males and 10 females), 18-affected carrying a full mutation (11 males and 7 females), and 2 NTM. An induced abortion was done in 3 cases (4.6%) due to another pathology (46,XY 18q-, Duchenne muscular dystrophy and cardiopathy). The CGG expansion was confirmed in all aborted fetuses of Fragile X families and newborns.. The sample should be obtained after 13 weeks of pregnancy when the methylation patterns are well established, then molecular prenatal diagnosis of fragile X syndrome is safe and reliable. This work was supported by grant Marato TV3 (98/310 IP.MM).

**Prenatal diagnosis of SMA-I utilizing a newborn screen dried blot specimen: A case report.** *K.B. Lescale, M.J. Erickson, K.M. Smith, F. Gilbert, M.F. Cabbad.* Department of Obstetrics and Gynecology, The Brooklyn Hospital Center, Brooklyn, NY.

Spinal muscular atrophy (SMA) is a disorder characterized by degeneration of the anterior horn cells of the spinal cord. It is a lethal, autosomal recessive disease with a frequency of approximately 1/20,000. The disease is classed into three types with Type I presenting in early infancy. Prenatal diagnosis for this disorder, by DNA testing, is available for at-risk couples. About 95% of affected individuals will have a homozygous deletion of exons 8 and/or 7 in the SMN gene on chromosome 5q and these can be detected by DNA analysis of amniocytes or chorionic villi. However, due to the reported incidence of intragenic mutations, *de novo* mutations, and the difficulty in distinguishing between a carrier and an affected individual on prenatal testing, the protocol for prenatal diagnosis requires haplotyping of the affected child and the parents to provide the most accurate result. In this case, the affected child was deceased and haplotyping had not been performed prior to his death. The patient was a 39 y.o. whose second child was diagnosed, at a few weeks of age by DNA analysis, to have deletions of exons 7 and 8 and died at 5 months of age. The patient presented to genetics approximately 18 months after the child's death and follow-up with the original diagnostic lab revealed that DNA was stored for a 12 month period only. In this case, the New York State newborn screen dried blood spot specimen was available. Haplotyping analysis of the dried blood spot and the parental bloods identified informative markers for linkage so the prediction of disease status would be possible for this couple in future pregnancies. Through the use of CVS, disease status was confirmed in two subsequent pregnancies. To our knowledge this is the first reported prenatal diagnosis of SMA-I utilizing the newborn screen blot of a deceased affected individual.

**Perinatal Hospice: An important option for families continuing pregnancies following the diagnosis of lethal conditions.** *R.J. Hopkin<sup>1</sup>, M.E. Walker<sup>1</sup>, H.M. Saal<sup>1</sup>, E.K. Schorry<sup>1</sup>, L. Tucker<sup>2</sup>*. 1) Dept Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) StarShine Hospice, Children's Hosp Medical Ctr, Cincinnati, OH.

Families faced with prenatal diagnosis of trisomy 18, 13, or other lethal conditions must make difficult decisions. For many pregnancy termination is not an acceptable option. Such families face the emotional burden of continuing a pregnancy while anticipating the death of their child. They often express feelings of confusion and anger, and may have little sense of control over what happens to them or their child. To address the needs of these families we have initiated a perinatal hospice program as a collaborative effort between the Division of Human Genetics and the StarShine Hospice at Children's Hospital in Cincinnati. The program includes genetic counseling, guidance for families in developing a detailed birth plan, coordination of communication between the family and their obstetric and pediatric care providers, coordination of home care and funeral arrangements, provision of comfort care to the affected infants, and grief counseling. Participating specialists include genetic counselors, hospice nurses, social workers, chaplains, and clinical geneticists. Of the 5 families enrolled in this program, 4 have delivered children with lethal chromosomal anomalies, and 1 a child with alobar holoprosencephaly. The perinatal hospice program empowers families in several ways: **1.** Families have tangible tasks to accomplish in the prenatal period. **2.** Writing a detailed birth and postnatal care plan aids in processing the natural history information provided through the genetic counseling. **3.** Decisions regarding management of apnea, feeding, and heart disease are made prospectively rather than in reaction to crisis. **4.** Follow up counseling and care are provided by professionals who have established relationships with the families. We believe this innovative program can serve as a model for the management of families who are continuing pregnancies following the prenatal diagnosis of lethal conditions.

**Prenatal marker chromosome identification utilizing the combination of G-banding, SKY and FISH.** *H.H. Heng<sup>1</sup>, J-W. Yu<sup>2</sup>, S. Ebrahim<sup>3</sup>, G. Liu<sup>1</sup>, C.J. Ye<sup>4</sup>, S. Bremer<sup>1</sup>, W. Lu<sup>1</sup>, M. Hughes<sup>1,3</sup>, S. Krawetz<sup>3</sup>, A. Johnson<sup>3</sup>.* 1) CMMG, School of Medicine, Wayne State Univ, Detroit, MI; 2) Eleanor Roosevelt Inst, Dever, CO; 3) Division of Reproductive Genetics, Ob/Gyn, Wayne State Univ, Detroit, MI; 4) SeeDNA Biotech Inc. Windsor, Ont, Canada.

The development of 24-color spectral karyotyping (SKY) has provided a more reliable method to identify marker chromosomes in prenatal diagnosis. However, due to the high cost of performing SKY and the current limited accessibility of facilities, it is not practical to perform SKY on every prenatal diagnostic sample. Therefore a compromised approach is needed to take advantage of the strengths of each method and yet is economically practical in the current clinical setting. We propose the use of routine G-banding for the preliminary examination of patients followed by SKY characterization if any marker chromosomes are detected by G-banding or if the patient is at risk. Verification if desired, would then be done with FISH once the origin of each element of the marker chromosome was determined by SKY. To demonstrate how this algorithm can be practically applied, we have analyzed amniocentesis fluid slides with the combination of G-banding, SKY and FISH tests. 20 cases were examined and the combination of tests was then evaluated for their effectiveness in determining a prenatal diagnosis and the results compared with the current conventional method of using G-banding alone then verifying with FISH. We have further tested new protocols that enable the re-use of conventional FISH or G/C-banded slides for the purpose of administering SKY. Our results show that the selective use of SKY serves as an important adjunct to current prenatal chromosomal analysis.

**Jumping translocation involving 21p in an amniotic fluid sample.** *S.L. Wenger<sup>1</sup>, O.C. Bleigh<sup>1</sup>, S.A. McAdoo<sup>2</sup>, B.K. Boyd<sup>2</sup>.* 1) Dept Pathology, West Virginia Univ, Morgantown, WV; 2) Dept Obstetrics, West Virginia Univ, Morgantown, WV.

Jumping translocations have been reported in individuals with chromosomal abnormalities, involving areas rich in repetitive DNA sequences. Recently, two tissues from spontaneous abortions were reported with jumping translocations (Levy et al., *Cytogenet Cell Genet* 88:25;2000). We report the first case identified in cultured amniocytes.

The patient was at risk for a fetus with Down syndrome due to maternal age (35 years). Amniotic fluid cells collected at 16 weeks gestation were set up and cultured. Only two colonies were identified as growing. Because of the limited number of cells, cultures were not analyzed by the coverslip method, but were trypsinized and harvested by the flask method. While many cells were normal, a few showed unbalanced rearrangements involving material attached to 21p. A total of 60 cells were analyzed; forty-four cells were normal 46,XX, six cells had an i(21)(q10), three cells were monosomy 21, two cells had 21ps-, and five single cells each had different additional chromosomal material on 21p. Both parents had normal karyotypes and a repeat amniocentesis at 19 weeks gestation for repeat karyotype of cultured amniocytes grew poorly. Only six metaphase cells could be analyzed; five normal and one with del(9)(q12), the latter most likely being tissue culture artifact. The last ultrasound at 26 weeks gestation did not identify any fetal abnormalities. The delivery date is early August. At that time, plans are to collect cord blood, umbilical cord for fibroblasts, and placenta. Each tissue will be examined for the jumping translocation, in order to determine if this finding is due to tissue mosaicism for a constitutional chromosome abnormality or pseudomosaicism in the amniocytes.

**Prenatal interphase analysis of translocation carriers using sub telomeric probes.** *M.J. Pettenati<sup>1</sup>, P. Mowrey<sup>2</sup>, S. Schwartz<sup>3</sup>, P.N. Rao<sup>4</sup>, J. Rosnes<sup>5</sup>.* 1) Dept Ped, Wake Forest Univ Sch Medicine; 2) Lab Corp of America; 3) Dept Human Genetics, Case Western Univ; 4) Dept Pathology, UCLA; 5) Dept Obs/Gyn, Wake Forest Univ Sch Medicine.

Reciprocal translocation carriers are fairly common. Most translocations have an intermediate risk (5-10%), some a fairly low risk (~1%), others no risk but a high likelihood of miscarriage, while a few have risks as high as 20% for an abnormal outcome. While reliable, rapid prenatal analysis using FISH for chromosomal aneuploidy is available; it may not be of primary concern to a translocation carrier.

Subtelomere probes now allow for the analysis of terminal chromosome rearrangements. We tested the ability of subtelomeric probes (ToTelVysion-Vysis) to rapidly detect prenatally the carrier status of a fetus when a parent carries a known chromosome translocation. Initially, the probes ability/hybridization efficiency was tested on balanced/unbalanced chromosome translocations on 12 control cases. Prospective prenatal interphase subtelomeric FISH analyses were performed on uncultured amniocytes and CVS (1) from 6 cases (5 reciprocal/1 Robertsonian). Appropriated translocation dependant probe sets were selected. Three of the cases were identified as being abnormal and later verified by cytogenetic analysis. The abnormalities included partial trisomies for 4q (amnio), 15q (amnio/CVS) and complete trisomy 13 (Robertsonian).

These analyses demonstrated the utility of FISH using these probes to rapidly and correctly identify balanced and unbalanced chromosome anomalies prenatally that can result from parental translocations. Caution is recommended with regards to insuring a careful cytogenetic analysis/review of the parental chromosome anomaly prior to selection of the probes. Subtelomeric FISH analysis represents a new rapid prenatal test available for known chromosome translocation carriers whose primary concern is the presence of a chromosomally balanced or unbalanced fetus.

**First trimester combined screening for chromosomal trisomies in Finland.** *M.A. Ryyanen<sup>1</sup>, M. Suonpaa<sup>4</sup>, M. Seppala<sup>4</sup>, M. Niemimaa<sup>1</sup>, A. Perheentupa<sup>1</sup>, P. Laitinen<sup>2</sup>, H.L. Kokkonen<sup>1</sup>, S.T. Heinonen<sup>3</sup>.* 1) Obstetrics & Gynecology, Oulu University Hospital, Oulu, Finland; 2) Clinical Chemistry, Oulu University Hospital, Oulu, Finland; 3) Obstetrics & Gynecology, Oulu University Hospital, Kuopio, Finland; 4) PerkinElmer Wallac, Turku, Finland.

**Background.** With current social trends toward smaller families and delays in childbearing, prenatal diagnosis has an important role in the management of most pregnancies. We wished to study in the first trimester the efficacy of separately and combining maternal serum test and fetal nuchal translucency measurement in screening for trisomies in Finland.

**Methods.** Written informed consent was obtained from all participating women. The screening test, PAPP-A and b-hCG and ultrasound were offered free of charge. Blood samples were analysed in PerkinElmer Wallac and the estimated risk for trisomies was calculated using Wallac 1T program.

**Results.** The participants comprised 4 489 volunteer pregnant women in eastern and northern Finland during the 10th-13th weeks of pregnancy in 1999-2000. Among the screenees there were 12 cases of trisomy 21 and 6 cases of trisomy 18 and 1 case of trisomy 13 and 15 each. Nuchal translucency was measured in 3 052 pregnancies. Eight cases of trisomy 21 and 5 cases of trisomy 18 and no cases of trisomy 13 or 15 were found in that group. 17.5% of the pregnant women were older than 35 years, a figure that conforms exactly to the average Finnish pregnancy statistics. Biochemical and nuchal translucency combined. In the screened population, a total of 238 women (5.3%) constituted the risk group for trisomies at 1:125 cut off level. Eleven out of twelve fetuses affected by Down's syndrome, 92%, and the 3 out of 6 fetuses with trisomy 18 were detected on the basis of the combined screening when algorithm for Down syndrome screening was used. Neither of trisomies 13 or 15 was detected.

**Conclusion.** This prospective study suggests that combined screening promises a better sensitivity than the current testing procedures used in Down's syndrome screening.



**Quantitative analysis of fetal DNA in maternal plasma in physiological pregnancies.** *G. Restagno<sup>1</sup>, L. Sbaiz<sup>1</sup>, F. Lagona<sup>5</sup>, M. Smid<sup>5</sup>, L. Maniscalco<sup>5</sup>, A. Ferrari<sup>5</sup>, G. Calori<sup>6</sup>, E. Viora<sup>2</sup>, M. Campogrande<sup>2</sup>, M. Pagliano<sup>3</sup>, M. Brizzolara<sup>3</sup>, T. Todros<sup>4</sup>, A. Vassallo<sup>7</sup>, M. Ferrari<sup>7</sup>, L. Cremonesi<sup>7</sup>.* 1) Dept Clinical Genetics; 2) Ecography, AO OIRM-S. Anna, Torino; 3) Cattedra A Obs Gynecol; 4) Unit materno-fetale, Univ Torino; 5) Dept Obs Gynecology; 6) Epidemiology; 7) Genetics and Molecular Diagnostics, H S Raffaele, Milano, Italy.

Recent studies demonstrated the presence of fetal DNA in maternal plasma, which represents a source of fetal genetic material obtained noninvasively. Before clinical application of this approach, it is crucial to assess whether fetal DNA can be retrieved from all women at any gestational age. We recruited 417 pregnant women with physiological pregnancy from the 6<sup>th</sup> to the 38<sup>th</sup> week of gestation. Fetal DNA quantification was carried out through real-time PCR on the SRY gene in male-bearing pregnancies. Mean and median fetal DNA concentration were 33.2 and 13.25 genome equivalents/ml of maternal plasma, respectively (range 0-361.2 ge/ml). Fetal DNA concentration increased with advancing gestational age and a statistically significant correlation was found between fetal DNA and gestational week ( $P=0.0001$ ). Fetal DNA analysis from maternal plasma displayed an overall 87% sensitivity, 100% specificity, 100% positive predictive value and 85% negative predictive value in fetal gender determination. We focused on two groups of pregnant women, the first one sampled at gestational ages ranging from the 11<sup>th</sup> to the 13<sup>th</sup>, the second from the 15<sup>th</sup> to the 17<sup>th</sup> week (most of prenatal diagnosis invasive procedures are performed in these periods). In the first group fetal DNA analysis had 60% sensitivity and 73% negative predictive value (mean and median fetal DNA concentration 30.7 ge/ml and 11.9 ge/ml respectively). In the second group, both sensitivity and negative predictive value were 84% (mean and median fetal DNA concentration 22.9 and 9.1). A number of demographic, anamnestic and clinical variables were examined. No statistically significant correlation was found between fetal DNA and maternal age, smoking habit, maternal blood group, Rhesus factor and previous obstetric history (particularly male-bearing pregnancies). Funded by Telethon.

**Enrichment and identification of fetal cells in maternal blood by the ISET technique: a non-invasive approach for prenatal diagnosis.** G. VONA<sup>1</sup>, C. BEROUD<sup>2</sup>, A. BENACHI<sup>3</sup>, JP. BONNEFONT<sup>4</sup>, A. QUENETTE<sup>2</sup>, S. ROMANA<sup>4</sup>, A. MUNNICH<sup>4</sup>, M. VEKEMANS<sup>4</sup>, Y. DUMEZ<sup>3</sup>, B. LACOUR<sup>2</sup>, P. PATERLINI-BRECHOT<sup>1,2</sup>. 1) INSERM Unit U370; 2) Biochemistry Unit; 3) Obstetric Unit; 4) Department of Genetics, Hopital et Faculté Necker, Paris, FRANCE.

Fetal cells circulate in the blood of pregnant women and therefore are of potential interest for early and non-invasive analysis. This approach has yet been hampered by the rarity of these cells (around 1 per ml). This difficulty can be overcome by enriching the blood sample in fetal cells, based on the size of epithelial (trophoblastic) cells.

Fetal cell enrichment was carried out using the ISET (Isolation by Size of Epithelial Tumoral cells) method. 2 ml of blood from 6 mothers carrying a male fetus were analysed at 11-12 weeks gestation. After morphological characterisation, 23 presumably-fetal single cells were microdissected. Their DNA was analysed with Y-specific (23 cells) and STR-specific primers (11 cells). In all mothers, we found a variable number of Y-positive cells (overall 15/23) while STR analysis showed a "biparental" pattern in 6/11 analysed cells. Taking into account the estimated number of fetal cells in the maternal blood (1 per ml, i.e. 1 per  $10^7$  WBC), we have obtained a 6.6- million fold enrichment (1 per 1.5 large isolated cell). Moreover, only one fifth of the single cell DNA amount was used for fetal origin assessment, leaving enough material for further genetic testing.

As a model, cultured tumoral cells (HuH6) were mixed with a blood sampling of a female control, enriched by ISET, microdissected and submitted to DNA analysis. We reproducibly detected a beta-catenin gene mutation by PCR, and we identified Y-positive cells by FISH in single HUH6 microdissected cells.

In conclusion, we have developed a reliable procedure to prove the fetal origin of circulating cells irrespective of the fetus gender, and to target a DNA analysis in these cells. This method seems to be a promising non-invasive approach for prenatal diagnosis of genetic disorders.

**It's Not Always Down's Syndrome - Chromosome Results After Amniocentesis.** *F. Stewart, B. Trainor, G.W. Smith, N.C. Nevin.* Dept Medical Genetics, Level A, Belfast City Hosp, Belfast, Northern Ireland.

The majority of women undergoing amniocentesis are hoping to find out whether or not their baby has Down's syndrome. We have recently audited the karyotypes obtained at amniocentesis in Northern Ireland over the last ten years. In the period 1990-1999 inclusive, 5516 amniocentesis samples were analysed by the prenatal cytogenetic laboratory in Belfast City Hospital. 5091 normal karyotypes were obtained. 87 showed normal variants of no clinical significance. In 62 cases cell culture failed and the amniocentesis was repeated. During this period 140 autosomal trisomies were detected: 89 trisomy 21; 39 trisomy 18 and 12 trisomy 13. A further 136 abnormal karyotypes were detected. A brief summary of these results is given here. 47 pregnancies had a balanced translocation all of which were clinically insignificant. 5 pregnancies had an unbalanced translocation, which would result in fetal abnormality. 7 pregnancies had triploidy. Other structural autosomal abnormalities were found in 41 cases. 36 pregnancies had a sex chromosome abnormality. Of these, 23 were 45, X Turners syndrome. Six had 47, XXX and one 47, XYY. Five had 47, XXY Klinefelters. The majority of cases with a clinically significant autosomal abnormality were tested because of an abnormal scan or an abnormal serum screen result. Our centre is considering the introduction of Fluorescent In Situ Hybridisation (FISH) to look at chromosomes 13, 18, 21, X and Y instead of conventional karyotyping. This would give rapid results on amniocentesis samples. Our findings suggest that the FISH technique would be unlikely to miss many clinically significant abnormalities provided that the ultrasound scan is normal. 32 abnormal results (non-trisomy) were obtained on women attending purely for maternal age. These included balanced rearrangements and sex chromosome abnormalities. Obstetricians need to be aware of the possibility of a non-Downs abnormal result. They need to arrange referral for genetic counselling in these circumstances to help these women make appropriate choices about the further management of their pregnancy.

**Prenatal diagnosis, outcome and imprinting in mosaic trisomy 16 pregnancies.** *P.J. Yong<sup>1</sup>, I. Barrett<sup>2</sup>, D.K. Kalousek<sup>3</sup>, W.P. Robinson<sup>2</sup>.* 1) Medicine; 2) Medical Genetics; 3) Pathology, University of British Columbia, Vancouver, Canada.

Statistical analysis was carried out on data from 174 prenatally diagnosed mosaic trisomy 16 pregnancies. The cases are from an ongoing study of trisomy mosaicism in Vancouver (n=62) and from other published reports (n=112). Additional cases with a paternal origin of trisomy, partial trisomy, or concomitant aneuploidy were excluded. Of the cases with information on pregnancy outcome (n=169), 67.4% resulted in live births, 12.4% in intrauterine deaths, and 20.1% in terminations. Among cases where weight or phenotype was known (n=87, n=134), the average weight at birth, intrauterine death or termination was 2.05 s.d. below the gestational-age corrected mean weight, and 46% had malformations. Since published cases and cases referred to the Vancouver study may be biased towards poorer outcome, these results should be interpreted with caution.

The presence of non-mosaic trisomy on CVS (direct or culture) was associated with lower weight compared a mosaic CVS finding (t=2.70, df=61, p=0.009). Likewise, the presence of trisomy on amniocentesis was associated with lower weight (t=4.15, df=66, p<0.001), and with an increased risk of major malformation (Fisher Exact test, n=103, p=0.010; RR=1.70). Of 66 cases of prenatal, postnatal or autopsy assessment of trisomy in blood, only one case tested positive. Of 40 cases of postnatal or autopsy assessment of trisomy in skin, 27.5% tested positive; however, the presence of trisomy in skin was not associated with any outcome variables. In contrast, among cases with determination of maternal uniparental disomy 16 (upd(16)mat) status (n=87), the presence of upd(16)mat was associated with lower weight (t=2.23, df=49, p=0.030), and with an increased risk of malformation (Fisher Exact test, n=79, p=0.022; RR=1.59). Male fetuses with upd(16)mat had a particularly poor outcome.

These results confirm that CVS and amniocentesis findings can be useful predictors of outcome, and neither cordocentesis or fetal skin biopsy is indicated after prenatal diagnosis of trisomy 16. Moreover, there is preliminary evidence for imprinting on chromosome 16.

**First trimester screening: the genetic implications on nuchal translucency (NT).** *E. Pergament, K. DeMarco, N. Ginsberg, R. Sabbagha.* Northwestern University Medical School, Chicago, IL.

**Purpose:** In conjunction with the NIH-sponsored, multicenter study on the efficacy of first trimester screening for Down syndrome, we undertook an analysis of the genetic implications of increased NT present in fetuses between 11 weeks 3 days and 14 weeks gestation. **Method:** The cytogenetic and/or phenotypic outcomes for patients at Northwestern (Chicago) for whom the NT exceeded 3.5mm were determined. **Results:** A total of 2,330 patients underwent first trimester screening; the NT of 21, or 0.9 percent, measured greater than 3.5mm (range: 3.6-7.5mm; median: 4.3mm). Nine, or 43 percent, were chromosomally abnormal (trisomies 13, 18, 20 and 21); two, or 10 percent, presented at birth with significant developmental disabilities; and, one, or 5 percent, exhibited a severe cardiac complex requiring transplantation. **Conclusions:** Adverse pregnancy outcomes involving chromosome aberrations, cardiac defects and syndromes associated with developmental disabilities occurred in nearly 60 percent of fetuses with a NT greater than 3.5mm. NT measurements applied to all pregnant women in the first trimester has the potential of causing a paradigm shift with regard to the timing, sensitivity and range of genetic and developmental disorders prenatally identified.

**Modification of Individual Risk with Second Trimester Ultrasound after Positive Maternal Serum Screen. D.**

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Maternal serum screen (MSS) for alpha-fetoprotein, unconjugated estriol and human chorionic gonadotropin between 15 and 20 weeks gestation is often utilized to modify individual risk for Down syndrome (DS). The determination of a positive result varies and is set by individual programs, but generally 5-8% of those taking the test will be offered an amniocentesis. Unnecessary amniocentesis is costly, both from a monetary and fetal loss perspective. To determine if 2nd trimester ultrasound following a positive MSS could more precisely predict which pregnancies were affected, ultrasound findings of those with a true positive MSS were compared with findings of those with a false positive MSS. Methods: A retrospective case-control study was carried out. From the provincial MSS data base, pregnancies with a positive MSS were chosen. Those confirmed with trisomy 21 were the cases whereas the next 4 consecutive positive MSS pregnancies without trisomy 21 were the controls. Only those who had an ultrasound prior to the karyotype results being known were included in the study. Ultrasound findings were compared in cases and controls and considered abnormal if there were structural abnormalities and/or at least one marker of aneuploidy. Results: 34 cases and 143 controls met the inclusion criteria. The sensitivity and specificity of prenatal ultrasound for detection of DS (most done at the BC Womens Health Centre) was 71%, (95% CI 55-86) and 83% (95% CI 77-89). An abnormal ultrasound in an MSS positive pregnancy increased the risk of trisomy 21 by a likelihood ratio (LR) of 4.20 (95% CI 2.8-6.4). A negative ultrasound decreased the risk by a LR of 0.35 (95% CI 0.21-0.60). MSS risk was modified retrospectively based on ultrasound results and although 30% of trisomy affected pregnancies had normal ultrasounds; all affected would have remained eligible for amniocentesis. Conclusion: In British Columbia, a targeted prenatal ultrasound can significantly modify the risk for trisomy 21 in a MSS positive pregnancy without reducing the over-all sensitivity of MSS.

**Biochip based enrichment of fetal cells from maternal blood.** *D.T. Yamanishi, P.J. Hujsak, J. Xu, Z. Yang, X-B. Wang, M. Huang, WP. Yang, L. Wu.* AVIVA Biosciences Corporation, San Diego, CA.

Previous studies have investigated different strategies for the isolation and enrichment of fetal cells from maternal peripheral blood as a method for non-invasive prenatal diagnosis. Our fetal cell enrichment system consists of a set of novel biochips that are capable of producing physical forces on the cells. Fetal cells from maternal blood were enriched on biochips based on physical and immunological characteristics. We have carried out separations on 20-40 mls of maternal blood from women during the first trimester of gestation, and identified variables such as antibodies, magnetic beads, and time of processing, that affected the sensitivity and enrichment for the separation procedure. In the initial studies, fetal cells were enriched using a density gradient followed by CD71 magnetic enrichment and dielectrophoresis separation. The enriched cells were put on a slide, and fetal cells were identified using antibodies to gamma and epsilon hemoglobin and X and Y chromosome interphase FISH. Our recent studies have utilized a physical separation unit as a replacement for the density gradient. Our data demonstrates that biochip based separation is an effective approach for enriching rare fetal cells from maternal blood samples.

**Fetal Hydranencephaly Following Exposure to Mifepristone.** *D.N. Saller Jr<sup>1</sup>, M.S.T. Qureshi<sup>2</sup>, C.J. Peterson<sup>1</sup>, P.C. Eggers<sup>2</sup>, E.A. Schaff<sup>2</sup>, R.K. Miller<sup>2</sup>.* 1) OB/GYN, Div Maternal-Fetal Med, Strong Memorial Hosp, Rochester, NY; 2) OB/GYN, University of Rochester School of Medicine, Rochester, NY.

A 26 year old Gravida 4 Para 1111 female entered a medical termination of pregnancy trial at 5 weeks and 4 days of gestation by last menstrual period confirmed by ultrasound. She took 200 mg of mifepristone (formally known as RU-486) orally. She subsequently experienced heavy bleeding and did not take the misoprostol prescribed to complete the termination. She was then lost to follow-up, under the impression that her termination was completed. She presented one week later with complaints of nausea. A viable pregnancy was confirmed by ultrasound and the patient opted to continue the pregnancy. Transabdominal and transvaginal ultrasound at 11 weeks revealed a simple cystic mass occupying the entire intracranial space. The findings suggested hydranencephaly. The patient proceeded with surgical pregnancy termination, but pathologic evaluation was not performed. Hydranencephaly is the absence of cerebral hemispheres. It is thought to occur through an early insult to the developing brain, usually due to an ischemic insult or overwhelming viral infection. The timing of such an ischemic insult would fit temporally with the exposure to mifepristone. Mifepristone is a progesterone antagonist, which increases myometrial activity and disrupts the corpus luteum. Additionally, it causes decidual necrosis and detachment of trophoblast, and thereby interferes with production of placental chorionic gonadotropin, lactogen and progesterone. As mifepristone is used for pregnancy termination, limited information is available as to its teratogenic effects. We are aware of eight reported cases of ongoing pregnancy following exposure to mifepristone. One previous case of congenital malformations following exposure to mifepristone has been reported to include sirenomelia (caudal regression) associated with facial clefting. These two cases demonstrating an association of congenital malformations with in utero exposure to mifepristone suggest that genetic counseling and prenatal diagnosis should be considered in ongoing pregnancies following exposure to mifepristone.



**Prenatal diagnosis from fetal urine in fetuses with bladder outlet obstruction.** *A.E. Donnenfeld, D. Lockwood, T. Custer, A.N. Lamb.* Genzyme Genetics, Philadelphia, PA, Framingham, MA, Santa Fe, NM.

From 1995-2001 we identified 79 fetal urine samples obtained via percutaneous bladder aspiration submitted for prenatal chromosome analysis. On 4 occasions, chromosome analysis from fetal urine was cancelled prior to laboratory evaluation. 75 fetal urine specimens underwent laboratory examination. Traditional cytogenetic analysis was attempted on all 75 specimens. FISH was attempted on 31 samples. Both cytogenetic and FISH analysis were performed according to standard techniques, utilizing the same protocol as for amniotic fluid samples. Traditional cytogenetic analysis was successful on 71/75 (95%) of samples. The absence of cell growth in culture occurred on 4 occasions. FISH evaluation was successful on 20/31 (65%) of specimens. In one case, traditional cytogenetic analysis was unsuccessful but FISH was informative. Chromosome information was obtained on 72/75 (96%) of fetal urine specimens. Of the 11 unsuccessful FISH studies, all were due to insufficient cells. The mean gestational age was 20.3 weeks (range 15-32) and the mean sample volume was 22.5 mL (range 4.5-50). The mean turn-around-time was 8 days (range 5-14) for cytogenetic analysis and 2 days (range 1-5) for FISH. Chromosome abnormalities were detected in 6/78 (7.7%) pregnancies with bladder outlet obstruction. There were two trisomy 21's, one trisomy 13, one interstitial deletion of chromosome 13, one unbalanced translocation (20p+), and one apparently balanced de novo reciprocal translocation. Conclusion: traditional cytogenetic analysis from fetal urine is readily achievable with a high success rate, in our series, 95%. The addition of FISH resulted in a 96% success rate. FISH was unsuccessful in one third of cases due to insufficient cells. However, the rapid turn-around-time achievable with FISH allows for expedient clinical management of bladder outlet obstruction, including placement of a vesico-amniotic shunt, when applicable, approximately 6 days sooner than would be possible if waiting for traditional cytogenetic results to become available. For this reason, FISH is warranted in the management of fetal bladder outlet obstruction. A 7.7% chromosome abnormality rate was identified.

**A derived benefit of the FASTER Trial: early sonographic detection of fetal anomalies.** *S.M. Carter<sup>1</sup>, S.J. Gross<sup>1</sup>, A. Monteagudo<sup>2</sup>, F. Malone<sup>3</sup>.* 1) Reproductive Genetics, Montefiore Medical Ctr, Bronx, NY; 2) New York University Medical Center, New York, NY; 3) Columbia-Presbyterian Medical Center, New York, NY.

The FASTER trial is a multicenter, US-based study to assess the efficacy of first trimester screening using nuchal translucency measurements and maternal serum analysis of free BHCG and PAPP-A to screen for Down syndrome. To date more than 15000 patients have been screened. Ultrasound (transabdominal or transvaginal) and serum marker analysis are performed from 10 weeks 4 days to 13 weeks days of gestation (CRL 36-79 mm). A suggested benefit of this study is the early detection of pregnancy loss, twins, and fetal anomalies. By protocol, women who are identified with any of these conditions are excluded from the FASTER study and are managed appropriately. The aim of our study was to review the diagnosis and pregnancy outcome of the these excluded cases in the setting of first trimester prenatal sonography. From more than 216 patients deemed ineligible, we have identified 5 cases of cystic hygroma, 2 cases of holoprosencephaly, 1 case of severe multiple congenital anomalies, and 1 set of monozygotic monoamniotic twins with severe cord entanglement. Early identification of these conditions allowed for cytogenetic analysis, further consultation, and termination for lethal disorders. Additionally, early evaluation of the fetus allowed for more accurate assessment of small for gestational age fetuses in the 3rd trimester. We present the cases along with cytogenetic results and/or pregnancy outcomes.

**Nuchal Translucency: a matter of transient jugular lymphatic distension.** *M.C. Haak<sup>1</sup>, M.M. Bartelings<sup>2</sup>, D.G. Jackson<sup>3</sup>, S. Webb<sup>4</sup>, A. Gittenberger de Groot<sup>2</sup>, J.M.G. van Vugt<sup>1</sup>.* 1) Obs/Gyn, VU medical center, Amsterdam, the Netherlands; 2) Anatomy/Embryology, Leiden Medical Center, the Netherlands; 3) Institute of Molecular Medicine, John Radcliff Hospital, Oxford, UK; 4) Anatomy & Developmental Biology, St.George Hospital, London, UK.

**BACKGROUND:** Ultrasonographic measurement of the fetal nuchal translucency (NT) is a widely used screening method for chromosomal abnormalities in pregnancy. This screening method is used in many countries to identify fetuses that are at risk for having Downs syndrome. When the fetal karyotype is proved to be normal the increased NT is still associated with a diversity of other fetal malformations. The mechanism explaining the abnormal fluid accumulation and the transient nature of NT remain unexplained. **METHODS:** The nuchal regions of human fetuses and trisomy 16 mouse embryos were examined for (lympho)vascular abnormalities using immunohistochemical markers against lymphatic (LYVE-1) and smooth muscle (1A4) and endothelial (CD34) cells. Additionally an ultrasonographic study was carried out on 17 fetuses with an increased NT. **RESULTS:** In both human and mouse specimens we found a mesenchyme lined cavity within the posterior nuchal region as well as bilaterally enlarged jugular LYVE-1 positive lymphatic sacs. The persistence of jugular lymphatic sacs was also confirmed by ultrasound in 14 human fetuses with increased NT. **CONCLUSION:** Our findings indentify the cause of increased NT as mesenchymal oedema in the presence of distended jugular lymphatic sacs, detected by the hyaluronan receptor LYVE-1. The delayed organisation and connection of these lymphatic sacs to the venous circulation might explain the transient nature of NT. Once lymphatic organisation has been established the excess of nuchal fluid can drain away leading to a disappearance of the enlarged nuchal translucency.

**Post-zygotic origin of complete maternal chromosome 7 isodysomy and consequent loss of placental PEG1/MEST expression.** *M. Miozzo<sup>1</sup>, F.R. Grati<sup>1</sup>, G. Bulfamante<sup>3</sup>, F. Rossella<sup>1</sup>, M. Cribiù<sup>3</sup>, T. Radaelli<sup>2</sup>, B. Cassani<sup>3</sup>, T. Persico<sup>4</sup>, E. Ferrazzi<sup>4</sup>, I. Cetin<sup>2</sup>, G. Pardi<sup>2</sup>, G. Simoni<sup>1</sup>.* 1) Medical Genetics, Dept. of Medicine, Surgery & Dentistry, University of Milan, S.Paolo School of Medicine, Milan, Italy; 2) Obstetrics and Gynecology, Dept. of Medicine, Surgery & Dentistry, University of Milan, S.Paolo School of Medicine, Milan, Italy; 3) Pathology, Dept. of Medicine, Surgery & Dentistry, University of Milan, S.Paolo, Milan, Milan, Italy; 4) Obstetrics and Gynecology, Biomedical Science Institute L.Sacco, University of Milan, Milan, Italy.

Maternal UPD of chromosome 7 is associated with pre- and post-natal growth retardation (IUGR, PNGR) and Silver-Russell syndrome (SRS [ MIM 180860]). We report a case of IUGR in a new-born with SRS stigmata. Using combined haplotyping and cytogenetic-FISH studies we characterised the lymphocytes, umbilical cord and four placental cotyledons. The results are consistent with complete maternal isodisomy 7 and trisomy 7 mosaicism of post-zygotic origin. The trisomic cell line was prevalent in trophoblast cells from two placental cotyledons. Trisomy 7 of post-zygotic origin is a frequent finding, but maternal isodisomy 7, due to trisomic rescue has never been reported. PEG1/MEST expression was evaluated on placenta cDNA and a specific transcript was revealed only in the cotyledons with a high percentage of trisomic cells and the presence of the paternal chromosome 7 contribution, but not in the placental biopsies with maternal isodisomy 7. The histological features of the four placental fragments revealed that isodisomy 7 correlates with a pattern of cotyledonary hyper-ramification due to an increase of the branching angiogenesis, which could be the result of a defect of angiogenesis caused by the absence of PEG1 product. The severe hypo-ramification of the two cotyledons, showing trisomy 7 mosaicism, may be due to the triplicate dosage of genes on chromosome 7. The delayed fetal growth could be the phenotypic effect of the imbalance between imprinted and non-imprinted genes on chromosome 7 in the fetus or the result of abnormal placental function during pregnancy.

**Identification of a second mechanism of X-linked recurrent spontaneous abortion (RSA): Tissue-selective cell disadvantage.** *C.A.G. Scacheri<sup>1,2</sup>, M.C. Lanasa<sup>3</sup>, J.M. Giron<sup>1</sup>, M.M. Thouin<sup>1</sup>, C. Romig<sup>2</sup>, W.A. Hogge<sup>3</sup>, E.P. Hoffman<sup>1,2</sup>.* 1) Children's National Medical Center, Washington, DC; 2) The George Washington Univ, Washington, D.C; 3) Magee-Womens Hospital, Pittsburgh, PA.

Approximately 2% of women have highly skewed X-chromosome inactivation (XCI), with preferential use of one X chromosome in >90% of cells studied. We previously showed that skewed XCI patterns are heritable (Pegoraro et al (1997)), and found concordance in skewing patterns between the primary blood and oral mucosal cells in individuals tested. In a recent case-control study, we showed that a higher proportion of women experiencing first trimester RSA displayed highly skewed X inactivation (14%) (Lanasa et al, 2001). Here, we report two newly ascertained families with apparent X-linked recurrent spontaneous abortion (RSA), where the RSA trait co-segregates with extremely skewed X inactivation in blood nucleated cells, and with random X inactivation patterns in oral mucosal cells. Also, losses in these families occurred in the second trimester of pregnancy, and all sexed cases were male. In one family, polyhydramnios was noted in several pregnancies, and one male fetal demise had mild ventriculomegaly. The other family shows no apparent phenotypic findings. There was no history of immune deficiency or other symptoms suggesting XSCID or other hematologic disorder. In both families, the maternally inherited X-chromosome is preferentially inactivated in peripheral blood, which is consistent with an X-linked lethal gene that shows growth disadvantage in a subset of cells in female carriers. In conclusion, we have now identified two different types of X-linked lethal traits: (1) those showing complete skewing of X-inactivation in all tissues in female carriers, with first trimester loss of males inheriting the trait; and (2) those showing complete skewing in blood, but not oral mucosal cells in female carriers, with second trimester loss of males inheriting the trait. Female carriers are asymptomatic in both types of X-linked recessive RSA. These findings further encourage molecular testing of recurrent aborters for X inactivation abnormalities (see <http://www.cnmcxcis.org>).

**Aminoglycoside antibiotics ameliorate exon skipping associated with a nonsense mutation.** S. Tsujino, T. Miyamoto, N. Kanazawa. Inherited Metabolic Diseases, National Inst Neuroscience, Tokyo, Japan.

Exon skipping is occasionally associated with premature nonsense codons amid exonic sequences. The mechanism is, however, as of yet unclear. On the other hand, it is known that aminoglycoside antibiotics allow the reading of full coding sequences through premature termination codons, and this phenomenon may be applied for therapy of human genetic diseases with premature terminal codons. We previously reported an exonic nucleotide change C-to-T, resulting in a nonsense mutation R179X, in the mitochondrial ornithine transporter gene, *ORNT1*, of a patient with hyperornithinemia, hyperammonemia and homocitrullinuria (HHH) syndrome (MIM 238970). This mutation leads to skipping of exon 4, which contains the nucleotide change. In this report, we observed by RT-PCR that G418 ameliorate the exon skipping due to R179X in cultured skin fibroblasts from the patient. Furthermore, G418 partially restored the function of ornithine transporter in the cells, estimated by a <sup>14</sup>C-ornithine incorporation assay, indicating not only amelioration of the exon skipping but also read-through of the premature termination codon, because only the return of exon 4 resuscitates the nonsense codon and a truncated *ORNT1* product should not function more than that from the exon skipping mRNA. Although aminoglycosides are known to inhibit self-splicing of prokaryotic group I introns, our results are the first to show that aminoglycosides have an effect on RNA splicing in human cells.

**Preclinical Treatment Approaches in Succinic Semialdehyde Dehydrogenase (SSADH) Deficiency.** *M. Gupta<sup>1</sup>, H. Bartels<sup>1</sup>, R. Greven<sup>1</sup>, H. Senephansiri<sup>1</sup>, B. Hogema<sup>1,2,3</sup>, C. Jakobs<sup>3</sup>, W. Froestl<sup>4</sup>, T. Bottiglieri<sup>5</sup>, O.C. Snead<sup>6</sup>, M. Grompe<sup>1</sup>, K.M. Gibson<sup>1</sup>.* 1) Molecular and Medical Genetics, Oregon Health Sciences Univ, Portland, OR; 2) Erasmus Univ, Rotterdam, The Netherlands; 3) Clin Chem, Free Univ, Amsterdam, The Netherlands; 4) Novartis Pharma, Basle, Switzerland; 5) Inst of Metab Dis, Baylor Univ Med Center, Dallas, TX; 6) Dept Neurol, Hosp for Sick Children, Toronto, Ontario, Canada.

Gamma-hydroxybutyrate (GHB), increasingly consumed as an illicit euphoric agent with potentially serious side effects, is the biochemical hallmark of human SSADH deficiency. To explore preclinical treatment approaches, our laboratory developed SSADH-deficient mice. These mice have up to 100-fold increase in tissue GHB levels, are developmentally delayed and atactic, suffer from seizures and die 3 weeks postnatally. We attempted pharmacologic rescue with vigabatrin (50-800 mg/kg), CGP-35348 (a GABAB receptor antagonist; 100-2000 mg/kg) and taurine (2000-5000 mg/kg). Taurine was selected based upon correlation of death with the weaning period. All interventions led to significant survival (22-33%;  $p < 0.05$ ). Simultaneously, we assessed locomotor and behavioral activity to monitor clinical efficacy. Analysis of seven independent locomotion variables and fifteen behavioral paradigms based upon the Irwin test battery indicated that vigabatrin > taurine > CGP 35348 in relation to clinical efficacy. CGP 35348 appeared to exacerbate hyperactivity and agitation associated with stress inducing situations in SSADH-deficient mice. Whereas vigabatrin has been linked to visual field defects, taurine is readily available, well tolerated, and possesses numerous neuroprotective features and receptor interactions. Taurine may have therapeutic relevance for SSADH deficiency and illicit GHB consumption (supported by: NS 40270 and March of Dimes #1-FY00-352).

**Huntingtin inhibits acetyltransferases and histone deacetylase inhibitors suppress *in vivo* pathogenesis.** J.S.

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Transcriptional dysregulation and loss of function of the transcriptional co-activator CREB-binding protein (CBP) have been implicated in the pathogenesis of Huntington's Disease (HD) and other neurodegenerative diseases caused by proteins with expanded polyglutamine repeats. We find that the polyglutamine-containing domain of Huntingtin, Httex1p, directly interacts with the acetyltransferase domains of CBP and p300/CBP Associated Factor (P/CAF), and inhibits acetyltransferase activity *in vitro*. Expression of Httex1p in cultured cells reduces the level of acetylated histones H3 and H4, and this reduction can be reversed by administration of histone deacetylase (HDAC) inhibitors. *In vivo*, HDAC inhibitors arrest progressive polyglutamine-induced neuronal degeneration and reduce lethality in a *Drosophila* model of polyglutamine diseases. These findings raise the possibility that HDAC inhibitor therapy may prove efficacious in slowing or preventing the progressive neurodegeneration seen in HD and other polyglutamine repeat diseases, even after onset of disease symptoms.



**Transplantation of precursor cells for hepatobiliary cells from the salivary gland as treatment of Wilson disease in LEC (Long-Evans cinnamon) rats, rat model of Wilson disease.** *K. Okumura<sup>1</sup>, K. Nakamura<sup>1</sup>, Y. Kimoto<sup>1</sup>, K. Matsumoto<sup>2</sup>, K. Terada<sup>3</sup>, T. Sugiyama<sup>3</sup>, F. Endo<sup>1</sup>.* 1) Department of Pediatrics, Kumamoto University School of Medicine; 2) Institute for Animal Experimentation, Tokushima University School of Medicine, Tokushima, Japan; 3) First Department of Biochemistry, Akita University School of Medicine, Akita, Japan.

Cell transplantation is an effective therapy of inherited diseases, and the liver is one target for stem cell therapy. Hepatic oval cells induced by chemical carcinogens are candidates of adult liver stem cells. However, identification of stem cells in the adult liver remained to be attained. Recent studies indicated that there are stem cells for hepatocytes in a fraction of hematopoietic stem cells. We attempted to identify precursors of hepatobiliary cells in the salivary gland, and cell transplantation was done in LEC rats, a model for Wilson disease. Male SD rats or LEA (Long-Evans agouti) rats were used as donors. Salivary glands were treated by collagenase / hyaluronidase digestion, and prepared into single cell suspensions. Recipient female rats were administrated 2AAF (2-acetylaminofluorene), according to the 2AAF/PH (partial hepatectomy) protocol. The salivary gland cells were transplanted immediately after 2/3 partial hepatectomy. We detected donor cell derived oval cells at 1-2 weeks after transplantation as determined. In the recipient liver, donor-derived hepatocytes and bile duct epithelial cells were visible from 2 weeks after the transplantation, as determined by in situ hybridization of the Y-chromosome sry gene. Alternatively, we isolated and cultured epithelial cells from the salivary gland of male LEA rats, and the cultured cells were transplanted into hepatectomized female LEC rats. Thus, the cultured cells transdifferentiated into hepatocyte and bile duct epithelia. Stem cell-like cells for the liver apparently present in the salivary gland.

**Bone marrow transplantation to treat liver damage in LEC rats, a model of Wilson disease.** *Y. Kimoto<sup>1</sup>, K. Nakamura<sup>1</sup>, K. Okumura<sup>1</sup>, K. Matsumoto<sup>2</sup>, T. Yamamoto<sup>3</sup>, F. Endo<sup>1</sup>.* 1) Department of Pediatrics, Kumamoto University School of Medicine, Kumamoto, Japan; 2) Institution for Animal Experimentation, Tokushima University School of Medicine, Tokushima, Japan; 3) Division of Molecular Pathology, Graduate School of Medical Science, Kumamoto University School of Medicine, Kumamoto, Japan.

Bone marrow cells can transdifferentiate into different cell types. Recent studies demonstrated that bone marrow derived cells can transdifferentiate into hepatocytes in rodents and in humans. We used the LEC (Long-Evans Cinnamon) rat as a model of Wilson disease. LEC rat carries a mutation on *Atp7b*, a homologue of human *ATP7B* and the clinical and biological features of LEC rats resemble findings in patients with this disease, including such ceruloplasmin deficiency and hepatitis. We attempted to determine if bone marrow derived cells would overcome the clinical and biochemical abnormalities seen in LEC rats. We used male LEA (Long-Evans agouti) rats as an immunocompatible donor for the cell transplantation. After the bone marrow transplantation, recipient female LEC rats were killed. Genomic DNA from donor cells was PCR detected in bone marrow and liver. In situ hybridization revealed that cells derived from donor rats were expanded in the liver. Hence, bone marrow transplantation for treatment of Wilson disease warrants further attention.

**Enzyme replacement therapy with recombinant alpha-galactosidase A in Fabry disease : Preliminary experience in patients with end-stage renal disease or post transplant.** *D.P. Germain, P. Bonfils, P. Boutouyrie, N. Brion, M. Froissart, C. Jacquot, X. Jeunemaitre, C. Orssaud, P.F. Plouin, B. Richalet, J.F. Toussaint, J.N. Fiessinger.* Dept Genetics, European Hospital Georges Pompidou, Paris, France.

**Background:** Fabry disease, lysosomal alpha-galactosidase A (a-Gal A) deficiency, results from the progressive accumulation of globotriaosylceramide (GL3) and related glycosphingolipids. Involvement of the vascular endothelium leads to early demise of classically affected patients from microvascular disease of the kidney, heart, and brain. A Phase 3 multi-center, randomized, double-blind, placebo-controlled study, recently showed that recombinant human a-Gal A (r-haGalA) replacement completely cleared accumulated GL3 from the vasculature of the kidney, skin and heart in 98%, 95% and 75% respectively, of the patients evaluated (Eng et al., N Engl J Med 2001). Patients with serum creatinine > 2.2 mg/dL, on dialysis, or transplanted were excluded from the Phase 3 trial, and have not previously been evaluated following enzyme replacement therapy. **Patients and Methods:** R-haGal A safety and efficacy were evaluated in an open-label study of 8 Fabry patients who received 0.8-1.2 mg/kg of r-haGal A (Fabrazyme, agalsidase beta) intravenously at 0.25 mg/min biweekly for 16 weeks (8 infusions). Six patients had advanced disease including 4 patients post renal transplant, one patient on peritoneal dialysis, and one patient on hemodialysis. Baseline assessments included medical history, physical examination, routine chemistries, plasma and urinary GL3, EKG, echocardiogram, and glomerular filtration rate. In addition, the following parameters which were not evaluated during the phase 3 trial were assessed: cardiac MRI, cerebral MRI, cochleovestibular involvement, and radial artery intima-media thickness. **Results:** No adverse events or infusion reactions were observed during the first 4 months of treatment. Immunologic evaluation including IgG seroconversion is ongoing. **Conclusion:** R-haGal A replacement appears safe in Fabry patients, including those on dialysis or post transplant. Efficacy will be evaluated at 6 months and one year.

**Analysis and Treatment of Pulmonary Pathology in the Acid Sphingomyelinase Deficient Mouse Model Of Niemann-Pick Disease.** *R.K. Dhami<sup>1</sup>, R. Gordon<sup>2</sup>, E.H. Schuchman<sup>1</sup>*. 1) Human Genetics, Mount Sinai School of Medicine, New York, NY; 2) Pathology, Mount Sinai School of Medicine, New York, NY.

Types A and B Niemann-Pick disease (NPD) are lipid storage disorders caused by the deficient activity of acid sphingomyelinase (ASM). Type B NPD is associated with pulmonary function decline and frequent respiratory infections. There is currently no treatment for either form of this disease. The ASM knockout (ASMKO) mouse is a model for NPD, but the lung pathology has not been adequately characterized. The overall goal of this study was to investigate pulmonary disease in the ASMKO mouse model and to compare the therapeutic effects of systemic (intravenous) delivery of recombinant ASM versus direct lung delivery (intratracheal). Using bronchoalveolar lavage, we found dramatically elevated levels of sphingomyelin (SPM) and inflammatory cells (mostly alveolar macrophages [Mf]) in the airspaces of the ASMKO mice. Furthermore, the ASMKO Mf were shown to have a reduced ability to produce superoxide, which could be corrected with ASM, and an increased propensity for cytokine secretion. Interestingly, we found no evidence of fibrosis in the ASMKO mice despite the presence of large numbers of inflammatory cells in the airways. In vitro studies demonstrated that the uptake of ASM by ASMKO alveolar Mf was reduced compared to alveolar Mf from normal mice, and also was reduced compared to ASMKO peritoneal Mf. This discrepancy in enzyme uptake may be a function of an alteration in mannose and mannose-6-phosphate receptors on the surface of SPM-loaded alveolar Mf from ASMKO mice as determined by competitive inhibition studies. In a direct comparison of lung-directed versus intravenous enzyme replacement in the ASMKO mice, we found that intravenous delivery was more effective in distributing enzyme to the lung tissue and reducing the numbers of alveolar Mf. We conclude that direct delivery of ASM to the lungs may not be a feasible strategy for the treatment of pulmonary pathology in Type B NPD due to cell surface alterations of lung Mf, but that intravenous delivery could be efficacious.

**Enzyme replacement therapy in Fabry disease: pathologic, biochemical, and immunologic results at 6 and 12 months of the Phase 3 extension study.** *R.J. Desnick*<sup>1,2</sup>. 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) The International Fabry Disease Study Group.

Fabry disease results from the deficient activity of  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) and subsequent pathologic accumulation of globotriaosylceramide (GL-3). Based on the results of a Phase 1/2 clinical trial (Eng et al. *Am. J. Hum. Genet.*, 68:711, 2001), a Phase 3 multicenter, multinational, randomized, double-blind, placebo-controlled trial was conducted with 58 classically affected patients receiving approximately 1 mg/kg of recombinant human  $\alpha$ -Gal A (r-haGal; produced by CHO cells by Genzyme Corporation) every two weeks for 20 weeks. The primary endpoint, the proportion of patients with clearance of accumulated GL-3 from renal interstitial capillary endothelial cells, was achieved ( $p < 0.001$ ) as were secondary endpoints for mean GL-3 clearance in the microvasculature of the skin ( $p < 0.001$ ) and heart ( $p < 0.001$ ). All 58 patients were treated with  $\alpha$ -Gal A in an ongoing open-label extension study. Infusion times have been reduced to approximately 3 hr. After 6 months, the placebo-crossover patients showed complete GL-3 clearance from their microvasculature (Eng et al., *N. Eng. J. Med.*, in press). Additional blinded pathologic assessments showed markedly reduced GL-3 levels from other renal cell-types (mesangial cells, glomerular capillary endothelium, non-capillary endothelium, and interstitial cells), as well as demonstrated reduction from the vascular smooth muscle cells, tubular epithelium, and podocytes, albeit at a slower rate. In addition, GL-3 reductions were observed in the endothelial cells of deep vessels of the skin, and from the cells of the perineurium. Plasma GL-3 levels were also measured. Importantly, the accumulated plasma GL-3 was cleared to below detectable limits in most patients at 6 and 12 months of the extension study. After 12 months of the extension study, 7/58 were seronegative, 8/58 patients who seroconverted were low-responders, and 67% of the remaining 43 patients reduced IgG titers at least two-fold, suggesting that patients may tolerize over time. These studies further demonstrate the safety and efficacy of  $\alpha$ -Gal A therapy for Fabry disease.

**A Phase I/II Randomized, Double Blind, Two Dose Group Study of Recombinant Human N-Acetylgalactosamine-4-Sulfatase (rhASB) Enzyme Replacement Therapy in Patients with Mucopolysaccharidosis (MPS) VI (Maroteaux-Lamy Syndrome).** *P. Harmatz<sup>1</sup>, C.B. Whitley<sup>2</sup>, K. Belani<sup>2</sup>, L. Waber<sup>3</sup>, R. Pais<sup>4</sup>, R. Steiner<sup>5</sup>, B. Plecko<sup>6</sup>, J. Simon<sup>1</sup>, J. Thompson<sup>7</sup>, J. Waterson<sup>1</sup>, E. Lammer<sup>1</sup>, R. Rowe<sup>1</sup>, S. Koseoglu<sup>1</sup>, R. Cohen<sup>1</sup>, H. Rosenfeld<sup>1</sup>, H. Gutierrez<sup>1</sup>, J. Hopwood<sup>8</sup>.* 1) Children's Hosp Oakland, CA; 2) U of Minnesota Medical School, Minneapolis; 3) U of Texas SW Med Center, Dallas, TX; 4) East Tennessee Children's Hosp, Knoxville; 5) Oregon Health Sciences U, Portland; 6) U Kidnerlinik Graz, Austria; 7) U of Alabama, Birmingham; 8) Women's and Children's Hosp Adelaide, Australia.

MPS VI is a lysosomal storage disease in which the affected patient lacks the enzyme N-acetylgalactosamine-4-sulfatase (ASB), leading to substrate accumulation in a wide range of tissues. This accumulation causes enlargement of liver and spleen, skeletal deformities, coarse facial features, upper airway obstruction, joint deformities, poor growth, progressive clouding of the cornea, communicating hydrocephalus, and heart disease. Death may occur in the second decade of life in more severely affected individuals. Currently there is no treatment for this disorder, other than symptomatic care and bone marrow transplant. Enzyme replacement therapy (ERT) with human recombinant ASB (rhASB) has been found to be effective in a feline model of MPS VI with few side effects. To assess the safety and efficacy in humans, a randomized, two-dose, blinded study of weekly treatment with rhASB is currently underway. Following a 2-week baseline evaluation period, subjects were randomized to high (1.0 mg/kg) or low (0.2 mg/kg) dose ERT which is administered intravenously once per week. Safety and efficacy assessments include cardiac evaluation, pulmonary function tests, overnight sleep study, joint range of motion, abdominal CT scan for liver volume and bone density, visual exam, exercise tolerance, routine laboratory analysis, and urinary glycosaminoglycans. Six subjects (3M, 3F; age range 7-16 years) have completed at least 12 weeks of therapy with no allergic or other serious adverse reactions noted. An analysis of the data of all subjects will be reported. (Sponsored by BioMarin Pharmaceutical).

**Growth improvement in response to enzyme replacement therapy (ERT) among children with Gaucher disease: The Gaucher Registry.** *P. Kaplan, H.C. Andersson, J. Charrow, E.H. Kolodny, P. Mistry, G.M. Pastores, B.E. Rosenbloom, C.R. Scott, R.S. Wappner, N.J. Weinreb.* International Collaborative Gaucher Group.

The Gaucher Registry is the largest database of patients (pts) with Gaucher disease (GD). This report describes the improvement in Z-scores (SD) and Annual Growth Rate (AGR) in response to ERT with alglucerase and imiglucerase among children with Type 1 GD.

Z-scores adjusted for age and gender were calculated using December 2000 Centers for Disease Control US Growth Charts. Two cohorts were analyzed:[1]193 prepubertal children (102 males up to age 9 yrs and 91 females up to 8 yrs),to minimize the bias of puberty.[2]AGR was assessed for 196 children (112 males and 84 females) by differences between heights measured at the beginning and the end of each 1-year period (increment in cm/12 mos). Velocity Z-scores adjusted for age and gender were calculated with reference to standardized distributions. Pts < 2.5 yrs were not included because of age limits in the velocity reference data.

Immediately prior to ERT, pts were almost two standard deviations below the expected height for an age-adjusted normal population (Z-score=-1.71). At 6 mos of ERT, improvements in growth were seen (Z-score=-1.41). At 30 mos through 48 mos, pts were <1 SD below the expected height for an age-adjusted normal population. The mean (SD) AGR after 12 mos of ERT was 8.0 cm (4.2). At the end of 24, 36, and 48 mos, the mean (SD) AGRs were 6.6cm (2.0), 7.0cm (1.8), and 6.3cm (1.9), respectively. The velocity Z-score after 12 mos of ERT was 1.02, approximately 1SD above the expected velocity for an age-adjusted normal population, confirming the catch-up growth reported by many physicians. After 48 mos,pts were <1SD above the expected velocity for an age-adjusted normal population. Expanding on previous studies in smaller cohorts of children, these data indicate that Z-scores and AGR for children with Type 1 GD, who are treated with ERT, approached the expected age-adjusted height for normal children. Furthermore, velocity Z-scores confirm that growth rates of Type 1 children treated with ERT often catch-up to the age-adjusted normal population.

**Alendronate disodium improves bone mineral density in adults receiving enzyme replacement therapy (ERT) for Gaucher disease.** *R.J. Wenstrup<sup>1</sup>, L. Bailey<sup>1</sup>, G.A. Grabowski<sup>1</sup>, S. Guo<sup>2</sup>*. 1) Human Genetics, Children's Hosp Research Fndn, Cincinnati, OH; 2) Wright State University, Dayton, OH.

Patients with Gaucher disease [glucocerebrosidase (GCase) deficiency] who have significant clinical manifestations are treated with injectable forms of the human enzyme. ERT results in remarkable decreases in lipid storage in liver, spleen, and bone marrow, but the severe generalized osteopenia present in many adult patients is often refractory to ERT. To determine whether the osteopenia present in most adults with Gaucher disease can be corrected by the bisphosphonate alendronate disodium (Fosamax<sup>TM</sup>) we performed a double blind, two-arm placebo controlled trial of alendronate (40 mg/day) on adults with Gaucher disease who had been treated at least 24 months with ERT. Thirty-six subjects (18-50 yrs.) receiving enzyme therapy (15-60 U/kg/q 2 weeks) were randomized to alendronate or placebo arms for up to 24 months; subjects in both trial arms received supplemental doses of calcium and vitamin D. Alendronate was well tolerated; there were seven dropouts [2 pregnant, 4 refused travel, 1 with GI symptoms (placebo)]. Both alendronate and placebo groups were 29% male. The mean age was 39.5 7.4 years in the alendronate group and 33.0 9.9 years in the placebo group. The mean baseline lumbar bone mineral density (BMD) was identical for both groups. For the primary study endpoint (lumbar BMD by dual energy X-ray absorptiometry), there were significant group differences. At six months DBMD at the lumbar spine was +0.035 0.023 grams/cm<sup>2</sup> for the alendronate group and +0.005 0.021 for the placebo group (p= 0.001). At 12 months, DBMD was +0.059 0.043 grams/cm<sup>2</sup> for the alendronate group and +0.009 0.037 for the placebo group (p= 0.006). Analysis of available BMD data from a more limited cohort who were followed for up to 24 months indicate that the group differences in lumbar BMD continue to increase. These data indicate that anti-resorptive therapy with bisphosphonates is a useful adjunctive therapy in combination with ERT for the treatment of Gaucher related osteopenia in adults.



**Preliminary findings in patients with juvenile Pompe's disease treated with recombinant human alpha-glucosidase from rabbit milk.** *L.P.F. Winkel<sup>1</sup>, H.J.M.P. van den Hout<sup>1</sup>, J.H.J. Kamphoven<sup>2</sup>, A.J.J. Reuser<sup>2</sup>, O.P. van Diggelen<sup>2</sup>, W.F.M. Arts<sup>1</sup>, P.A. van Doorn<sup>1</sup>, A.G. Vulto<sup>1</sup>, G. de Jong<sup>1</sup>, A.T. van der Ploeg<sup>1</sup>.* 1) Depts. of Pediatrics, Neurology, Hospital Pharmacy and Internal Medicine, University Hospital, Rotterdam, The Netherlands; 2) Dept. of Clinical Genetics, Erasmus University Rotterdam, The Netherlands.

Pompe's disease or Glycogen storage disease type II (GSDII) is an inherited myopathy. The characteristic lysosomal glycogen accumulation is caused by alpha-glucosidase deficiency. Childhood and juvenile forms of Pompe's disease present as a progressive muscle weakness with involvement of respiratory muscles. There was no treatment until now. Our research has focussed on the development of enzyme replacement therapy for Pompe's disease and has led to large-scale production of human recombinant alpha-glucosidase in milk of transgenic rabbits. In a phase II study this therapy was given to four patients with the most severe infantile form of Pompe's disease. Treatment appeared safe and effective (The Lancet 2000; 356: 397-8 and presented at the ASHG annual meeting in 2000). The alpha-glucosidase activity in muscle normalized, tissue morphology improved, cardiomyopathy resolved significantly and all 4 patients are still alive at age 2,5-3 years. In parallel, three patients with juvenile Pompe's disease received treatment. They were 12, 16 and 32 years old at start. Their clinical symptoms varied. They were all wheelchair bound. The two older patients were ventilator dependent and showed a significant deterioration of pulmonary function before start of treatment. After 1.5-2 years of treatment their pulmonary function has stabilized or improved. The alpha-glucosidase activity in muscle increased. The best improvement of muscle strength and function was obtained in the youngest and least affected patient. This patient started to walk after 4 years of wheelchair dependency. All patients report less fatigue and more energy. In conclusion, recombinant human alpha-glucosidase from rabbit milk has a positive effect in late onset Pompe's disease. There is all reason to continue the development of enzyme replacement therapy.

**A new approach to generate mice tolerant to recombinant human proteins.** *N. Raben<sup>1</sup>, A. Lee<sup>1</sup>, N. Lu<sup>1</sup>, K. Nagaraju<sup>1</sup>, Y. Rivera<sup>1</sup>, B. Yan<sup>1</sup>, A.A. McKinney<sup>2</sup>, E. Ponce<sup>2</sup>, W.M. Canfield<sup>2</sup>, P.H. Plotz<sup>1</sup>.* 1) ARB, NIAMS, NIH, Bethesda, MD 20892; 2) Novazyme Pharmaceuticals, Inc., Oklahoma City, OK 73104.

When knockout (ko) mice are used to test the efficacy and toxicity of recombinant human proteins, the animals develop antibodies to the human enzyme, precluding long-term pre-clinical studies. This has been a problem with a number of ko models, for example, the evaluation of gene or enzyme replacement (recombinant human acid alpha-glucosidase: rhGAA) therapies in a ko (GAA<sup>-/-</sup>) model of Pompe disease. In this disease, the lack of enzyme results in lysosomal glycogen storage, particularly in skeletal and cardiac muscle. In trying to rescue the phenotype of GAA<sup>-/-</sup> mice, we have expressed hGAA cDNA in the liver or skeletal muscle of these mice using a tetracycline-controllable system. Whereas high production of hGAA from the transgene in the liver in two lines led to secretion and metabolic cross-correction in muscle and heart, in one line with low GAA activity in the liver, (1-3% compared to wt), glycogen was cleared only in the liver but not in any other organ. Glycogen accumulation in the heart and skeletal muscle was similar to that in GAA<sup>-/-</sup> mice. Thus, this transgenic line retains the biochemical and morphological characteristics of the original ko mice, except for glycogen clearance in liver.

When HP-rhGAA (highly phosphorylated rhGAA, 1mg/kg) was injected i.v. weekly into GAA<sup>-/-</sup> mice or into animals in which the transgene expression was suppressed, high levels of antibodies were detected after 4 injections. In contrast, the low liver expresser mice did not develop antibodies or any adverse effects after 8 injections. This tolerant ko mouse model should be useful for evaluating the rhGAA in pre-clinical studies. Furthermore, mice in which the transgene expression was restricted to skeletal muscle were tolerant to the HP-rhGAA, too. The findings suggest that confining the expression of a transgene to a particular tissue or keeping its expression at non-therapeutic levels may prove a generally useful way for generating tolerant mouse models for other diseases or for other immunological studies.

**Gene therapy for lysosomal acid lipase deficiency in the mouse model.** *H. Du<sup>1</sup>, M. Heur<sup>1</sup>, J. Mishra<sup>1</sup>, J. Brannock<sup>1</sup>, D. Witte<sup>1</sup>, J. Rethmeier<sup>2</sup>, D. Ameis<sup>2</sup>, G. Grabowski<sup>1</sup>.* 1) Division of Human Genetics, Division of Pathology, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Dept Medicine, Univ. Hospital Eppendorf, Hamburg, F.R.G.

Lysosomal acid lipase (LAL) is an essential enzyme for the hydrolysis of triglycerides (TG) and cholesteryl esters (CE) in lysosomes. Its deficiency produces human two phenotypes: Wolman disease (WD) and cholesteryl ester storage disease (CESD). We created a LAL null (*lal*<sup>-/-</sup>) mouse model that closely mimics human WD and CESD. To test the potential for gene therapy, an adenoviral vector expressed human LAL (Ad-hLAL) was injected through tail vein to *lal*<sup>-/-</sup> mice at age of 2 months or 4 months with a single dose of viral particles  $3 \times 10^8$ /mouse or  $5 \times 10^9$ /mouse. Mice were sacrificed at 6, 20 and 47 days post-injection. All *lal*<sup>-/-</sup> mice injected with Ad-LAL or PBS survived. Compared to the PBS injected controls, the Ad-hLAL injected mice showed a reduction of plasma TG and CE levels, an increase in hepatic LAL activity of 272-fold at 6 days, and 62-fold at 20 days. Immunohistochemical staining with anti-hLAL antibody detected hLAL expression in hepatocytes and sinusoid lining cells in the liver, sinusoidal cells in the spleen, macrophages in the lung, and cortical cells in the adrenal glands. Hepatomegalies in *lal*<sup>-/-</sup> mice were reduced by 49% at 6 days, by 93-100% at 20 and 47 days in 2 months mice. The reductions of hepatomegaly in 4 months old mice were less significant (5.4%). Histological analyses of a variety of tissues showed reductions of lipid storage in hepatocytes and Kupffer cells in the liver and macrophages of the small intestine and the spleen. Biochemically, TG and cholesterol in liver and small intestine were reduced by ~75% at 6 days, ~95% at 20 days, and ~89% at 47 days in 2 months old mice. However, in 4 months old mice TG only was reduced in liver and small intestine by 50% and cholesterol concentration was unchanged. These studies provide "proof of principle" for adenoviral gene therapy in human WD and CESD.

**Correction of mitochondrial defects of human cells carrying mtDNA-encoded ND4 subunit by the yeast NDI1 gene.** *Y. Bai*<sup>1</sup>, *P. Hájek*<sup>1</sup>, *A. Chomyn*<sup>1</sup>, *E. Chan*<sup>1</sup>, *B. Seo*<sup>2</sup>, *A. Matsuno-Yagi*<sup>2</sup>, *T. Yagi*<sup>2</sup>, *G. Attardi*<sup>1</sup>. 1) Biology, Caltech, Pasadena, CA; 2) Division of Biochemistry, Department of Molecular and Experimental Medicine, The Scripps Institute, La Jolla, CA.

It is shown here that the gene for the single subunit, rotenone-insensitive and flavone-sensitive internal NADH-quinone oxidoreductase of *Sacchomyces cerevisiae* (NDI1) can completely restore the NADH dehydrogenase activity in mutant human cells that lack the essential mitochondrial DNA (mtDNA)-encoded subunit ND4. In particular, the NDI1 gene was introduced into the nuclear genome of the human 143B.TK<sup>-</sup> cell line derivative C4T, which carries a homoplasmic frameshift mutation in the ND4 gene. Two transformants, with a low or high level of expression of the exogenous gene, were chosen for a detailed analysis. In these cells, the corresponding protein is localized in mitochondria, and, in perfect correlation with its abundance, restores partially or fully NADH-dependent respiration, that is rotenone-insensitive, flavone-sensitive, and antimycin A-sensitive. Thus the yeast enzyme has become coupled to the downstream portion of the human respiratory chain. Furthermore, the P:O ratio with malate/glutamate-dependent respiration in the transformants is approximately two-thirds of that of the wild-type 143B.TK<sup>-</sup> cells, as expected from the lack of proton pumping activity in the yeast enzyme. Finally, while the original mutant cell line C4T fails to grow in the medium containing galactose instead of glucose, the high NDI1-expressing transformant has a fully restored capacity to grow in galactose medium. The present observations substantially expand the potential of the yeast NDI1 gene for the therapy of mitochondrial diseases involving complex I deficiency.

Program Nr: 2903 from the 2001 ASHG Annual Meeting

**Mutation-independent therapeutic strategies for Osteogenesis Imperfecta.** *G.J. Farrar, S. Millington-Ward, C. Allers, P. Conget, P.F. Kenna, P. Humphries.* Genetics, Trinity College Dublin, Dublin, Ireland.

Osteogenesis Imperfecta (OI) is a debilitating brittle bone disease which to date remains incurable. It is inherited in an autosomal dominant manner and is thought to affect 1/24,000 people. Over 150 and 80 mutations in the two type one collagen genes, COL1A1 and COL1A2 respectively, are known to give rise to the disease. Therefore developing mutation-specific gene therapies for this immensely heterogeneous disease may not be feasible. For this reason mutation-independent gene therapies for COL1A1 associated OI utilising hammerhead ribozymes are actively under development. A hammerhead ribozyme has been designed to suppress one allele of a common polymorphic variant of the Collagen 1A1 gene and therefore in principle this agent should be able to treat approximately 60% of patients with Collagen 1A1-linked OI. These ribozymes, administered via retroviruses, have now been tested in appropriate human cell lines and results from this study will be presented.

**AAV gene transfer in the GM2 gangliosidosis (Sandhoff disease) animal model.** *C. Bourgoïn<sup>1</sup>, P. Montanucci<sup>2</sup>, C. Emiliani<sup>2</sup>, R.A. Gravel<sup>3</sup>, L. Poenaru<sup>1</sup>, C. Caillaud<sup>1</sup>.* 1) Genetics, CHU Cochin, Paris, France; 2) Cellular and Molecular Biology, Perugia, Italy; 3) Research Institute, Montreal Children's Hospital, Montreal, 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, that encodes the hexosaminidases b-chain and results in a hexosaminidases A and B deficiency. Hex A and B are lysosomal hydrolases that are involved in ganglioside catabolism. Hex A is a heterodimer ab and Hex B a homodimer bb. Sandhoff disease predominantly affects the central nervous system, but also peripheral organs, such as liver. Specific AAV vectors, containing the human HEXA and HEXB cDNAs under the control of the CAG hybrid promoter, were constructed and their functionality was checked in vitro. They were then injected in the murine model of Sandhoff disease. Intramuscular administration of AAV-HEXB alone led to a restoration of Hex A and B activities in the injected muscle, but the coadministration of both AAVs led to a significantly enhanced Hex A specific activity. No secretion was observed in the peripheral blood system, as shown by serum and liver assays. AAVHEXB intravenous injections were performed in neonate Sandhoff mice. An increased hexosaminidases activity was found in the serum of the animals. It was stable for at least 2 months, reaching up to 30 % of the normal mouse activity. The liver hexosaminidases activity of the treated mice reached 60-90% of the normal. These results show that the liver is an efficient organ for the secretion of hexosaminidases, when transduced with AAV vectors. AAV-HEXB was also injected intracerebrally in *hexb*<sup>-/-</sup> neonates. Histological staining was performed with an artificial substrate coupled with a naphthol residue, showing a high enzyme activity in widely diffuse areas. Total hexosaminidases activity in the whole brain was restored to about the normal level, but Hex A reached only 20% of normal. The coadministration of HEXA and HEXB vectors will now be tested for its ability to provide therapeutic levels of both hexosaminidases A and B in the central nervous system.

**Analysis of SR proteins as therapeutic agents for SMA.** C.J. DiDonato<sup>1</sup>, P. Young<sup>2</sup>, C. Lorson<sup>2</sup>, R. Kothary<sup>1</sup>. 1) Ottawa Health Research Inst., Ottawa, Canada; 2) University of Arizona, Tempe, AZ, USA.

Mouse models of SMA have provided the most definitive evidence for the importance of full-length SMN (FL-SMN) transcripts and hence exon 7. They have demonstrated that SMN2 can rescue the embryonic lethality in *Smn*<sup>-/-</sup> mice, however high copy number of the SMN2 gene is required for complete rescue. This is consistent with the observation that the more copies of SMN2 present in SMA patients, the milder the disease. Thus, the presence of intact SMN2 gene(s) in SMA patients provides a natural target for therapeutic intervention. Identification of factors that enhance the production of FL-SMN from the SMN2 gene would be a major step towards the attenuation of the disease phenotype in SMA patients. One could search for agents that either upregulate the expression of SMN2 or suppress the alternative splice of SMN2 exon 7. Both would increase production of FL-SMN protein. We have focussed our efforts on the second approach.

A number of splicing factors, known as the SR proteins, have been shown to be involved in constitutive and alternative splicing. We have used a cell culture system and are characterizing two SR proteins that induce SMN2 exon 7 inclusion, Htra2-beta1 and SRp30C. The identification of these factors as modulators of SMN exon 7 alternative splicing is a crucial first step toward the possibility that they can be used to enhance the production of FL-SMN from the SMN2 gene of SMA patients. However, it is imperative that these factors be thoroughly characterized in a cell culture system and tested in the context of a whole animal. We have to be able to demonstrate that Htra2-beta1 and SRp30C can perform a "molecular rescue" of the SMN2 exon 7 alternative splice not only in SMA patient cell lines but also in a mouse model.

We will present our work on the cell culture characterization of Htra2-beta1 and SRp30C, and our progress towards creating transgenic mice that will be crossed to our SMN2 minigene mice that we have recently produced and characterized. This work has major implications on the *in vivo* therapeutic potential of these splicing factors.

**a- and b-sarcoglycan Delivery by AAV: Efficient Rescue of Muscle, but Differential Persistence of Gene**

**Expression.** *D. Dressman*<sup>1,2</sup>, *L. Liu*<sup>3</sup>, *E. Engvall*<sup>3</sup>, *K. Araiishi*<sup>4</sup>, *M. Imamura*<sup>4</sup>, *T. Sasaoka*<sup>4</sup>, *E. Ozawa*<sup>4</sup>, *E.P. Hoffman*<sup>1,2</sup>.  
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The sarcoglycanopathies are four autosomal recessive muscular dystrophies (LGMD 2D, 2E, 2C and 2F), caused by mutations in a, b, g and d sarcoglycan (SG) genes, respectively. Primary sarcoglycanopathies are estimated to account for 10-20% of muscular dystrophies in patients with normal dystrophin findings. To date, most adeno-associated virus (AAV) based gene delivery efforts in the muscular dystrophies have used the d-SG deficient hamster; however, primary d-sarcoglycanopathies are exceedingly rare in humans with only two patients reported in the US. We describe the construction and utilization of AAV vectors containing a- and b-SG, the most common of the human SG deficiencies. We show widespread genetic, biochemical and histological rescue of both a- and b-SG deficient knockout mouse muscle. We compared the persistence of expression in 36 AAV a-SG and 22 AAV b-SG injected mice. There was no evidence of decreased b-SG expression 133 days after injection. However, mice injected with a-SG showed dramatic loss of transduced myofibers at 28 days to 41 days post-injection. Loss of immuno-positive fibers was correlated with significant inflammation of macrophage >> CD4 or CD8 cells. To determine if the clearance of a-SG positive fibers was due to an immune response or was caused by a cytotoxic effect, immune deficient SCID mice were injected with either a- or b-SG AAV or sham injected. These experiments revealed that overexpression of a-SG causes significantly more cytotoxicity than either the sham injection or overexpression of b-SG. We hypothesize that overexpression of a-SG using a CMV-promoter-driven AAV construct results in cytotoxicity, while overexpression of b- or d-SG does not. This interpretation is consistent with emerging biochemical models of the hierarchical order of assembly of the SG complex and suggests that even closely related proteins may require different levels of expression to achieve tissue rescue.



**Functional correction of adult *mdx* mouse muscle using gutted adenoviral vectors expressing full-length dystrophin.** C. DelloRusso<sup>1,3</sup>, J. Scott<sup>3</sup>, D. Hartigan-O'Connor<sup>3</sup>, C. Barjot<sup>2</sup>, G. Salvatori<sup>2</sup>, A. Robinson<sup>2</sup>, S. Brooks<sup>1</sup>, J. Chamberlain<sup>3</sup>. 1) Departments of Physiology and; 2) Human Genetics, University of Michigan, Ann Arbor; 3) Department of Neurology, University of Washington, Seattle.

Duchenne muscular dystrophy is a degenerative lethal muscle disorder caused by mutations in the dystrophin gene. Adenoviral vectors are promising tools that may be used to express dystrophin in affected muscle. We have constructed 3 gutted adenoviral vectors devoid of all viral genes and containing a full-length human (HDys) or mouse (MDys and GEbDys) dystrophin cDNA driven by a muscle specific promoter. Virus was injected into 1 year old *mdx* mouse TA muscles and a novel lengthening contraction (LC) protocol was used to test for restored muscle function. This protocol reveals the high susceptibility of *mdx* TA muscles to contraction-induced injury; after 1 LC, wild type muscle force is reduced by 10% while *mdx* muscles show a 72% force loss. After 5 days, HDys injected muscles were significantly protected from injury and expressed high levels of dystrophin in contrast to sham injected control muscles. However, 25 days after injection, significant decreases in force generating capacity were detected. This loss of force was similar to that observed after injection of a first generation virus containing a LacZ transgene (CNb). MDys and GEbDys injected muscles demonstrated high levels of dystrophin protein expression and no functional defects after 25 days. In addition, MDys injected muscles were able to produce 62% of wild type force levels after 1 LC that, in contrast, reduced *mdx* and sham injected control muscles to 38 and 29% of wild type force, respectively. FACS sorting revealed the least amount of CD4+ and CD8+ cells in MDys injected muscles, 1/3 more immune cells in HDys injected muscles, and the highest number in muscles injected with CNb. Collectively, these data demonstrate 1) gutted adenoviral vectors are successful in transducing dystrophin in TA muscles of 1 year old *mdx* mice, 2) evidence for an immune response against human dystrophin that causes atrophy and loss of function, and 3) a partial functional correction of adult *mdx* mouse muscle after delivery of full-length mouse dystrophin.

**Pharmacodynamic and pharmacokinetic properties of retrovirus vector particles: Distribution after systemic delivery in vivo.** *G. Guibinga, C.-S. Park, T. Friedmann.* Pediatrics, UC San Diego, La Jolla, CA.

The development of safe, efficient and targeted gene transfer vectors for gene therapy will require increased knowledge of the pharmacological properties of the vectors and their mechanisms of target cell recognition and uptake. Retrovirus- and lentivirus vectors represent attractive gene transfer tools for in vivo gene delivery, but while the in vivo transducing properties of such vectors are coming to be characterized, little information is available on the physical distribution of the virus particles in vivo. Retrovirus and lentivirus preparations contain many non-infectious virus-like particles that may affect the toxicity and immunogenicity of the preparation and the resulting host response, but little information is available on the pharmacokinetic and pharmacodynamic properties of such retrovirus particles. It is therefore important to use methods independent of transgene expression to characterize the in vivo fate of retrovirus particles, their sites of accumulation and sequestration and their role in immune and non-immune related toxicity following systemic administration. In the current study, we have injected VSV-G pseudotyped MLV-based virus preparations expressing the lacZ transgene into Balb/c mice and have used immunohistochemical methods and deconvolution microscopy to characterize the distribution of viral gag protein as well as the sites of gene transfer by histochemical detection of lacZ activity. These studies were supported by grant HL64730 from the National Heart Lung and Blood Institute.

**Efficient cell attachment and infection by VSV-G pseudo-typed Moloney murine leukemia virus requires cell surface heparan sulfate.** *T. Friedmann, A. Miyanojara, G. Guibinga.* UCSD School of Medicine, La Jolla, CA 92093.

Infection of target cells by retrovirus is thought to be initiated by an interaction of a viral envelope protein with one or more specific and non-specific cell surface receptors followed by the fusion of the viral and the cell membranes and cell entry. We have shown that the initial attachment of retrovirus- and lentivirus vectors is independent of the known specific retrovirus receptors. To identify the cell membrane molecules involved in initial virus attachment, we have used deconvolution microscopy to examine the effect of glycosaminoglycans (GAG) on the initial attachment of envelope protein-free and VSV-G pseudotyped murine leukemia virus-based retrovirus vectors in vitro. Heparin effectively inhibits the attachment and infection of envelope-free and VSV-G pseudotyped retrovirus vector in human HT1080 cells. Furthermore, enzymatic digestion of heparan sulfate on the surface of target cells with heparinase I partially inhibits attachment and infection by both envelope protein-free and VSV-G pseudotyped retrovirus vector respectively. Digestion with chondroitinase ABC that specifically removes other GAG cell membrane produces no significant reduction of virus particle attachment. Interestingly, a CHO mutant cell line completely deficient in GAG (A-745) shows approximately 50% inhibition of attachment, but such cells are susceptible to infection by VSV-G pseudotyped retrovirus vectors. These data indicate the existence of redundant and alternative mechanisms of attachment of retrovirus particles that involve cell surface heparan sulfate and possibly other GAG molecules and phosphatidyl serine in conjunction with electrostatic interactions. The elucidation of the roles of these receptors in initial attachment and subsequent virus uptake and cell infection has obvious important implications for an understanding of vector pharmacokinetics and pharmacodynamics and for the design of targeted retroviral vectors. These studies were supported by grant HL64730 from the National Heart, Lung and Blood Institute.

**Gene therapy of Fanconi Anemia groups A and C by lentiviral vectors using mouse as an experimental model: Comparison with retroviral vectors.** *F. Galimi*<sup>1</sup>, *M. Noll*<sup>2</sup>, *Y. Kanazawa*<sup>1</sup>, *T.P. Lax*<sup>2</sup>, *R.L. Bateman*<sup>2</sup>, *A. D'Andrea*<sup>3</sup>, *I. Verma*<sup>1</sup>, *M. Grompe*<sup>2</sup>. 1) The Salk Institute for Biological Sciences, San Diego, CA; 2) Dept. of Molecular/Medical Genetics, Oregon Health Sciences University, Portland, OR; 3) Harvard Medical Institute, Boston, MA.

Fanconi Anemia (FA) is an autosomal recessive disorder characterized by birth defects, increased incidence of malignancy, progressive bone marrow failure and cellular hypersensitivity to DNA cross-linking agents. Bone marrow transplantation (BMT) is currently considered the treatment of choice for patients with FA, if a suitable donor is available. Therefore, gene therapy is an attractive alternative approach. In most genetic disorders, hematopoietic gene therapy would require correction of a large percentage of cells to be clinically effective. In FA we have potentially exploited the biology of the disorder itself. Previously, we have shown that bone marrow repopulation in FA mice was strong and rapid when direct selective pressure was applied in the form of low dose cyclophosphamide (CPA)/ablation (IR) which have previously been applied to FA patients. Recently, we used a VSVG pseudo-typed MMLV vector expressing the human FANCC cDNA to transduce *fancc*<sup>-/-</sup> marrow cells. We have shown that a single dose of CPA produced significant selection of retrovirally corrected HSCs. In the current study, we demonstrate the benefits of in vivo selection with third-generation lentivirus-derived vectors. These vectors might be the vectors of choice for HSC gene therapy since they allow efficient and stable gene transfer into quiescent stem cells with minimal manipulation of HSCs in comparison to the retrovirus. Our results show that a single dose of CPA produced significant selection of lentivirally corrected HSCs, with the integration of ~1 copy per genome. We have also generated the *fanca* knock-out mice and currently we are in process of carrying out similar in vivo selection procedures in these mice. Our preliminary data show that in vivo selection of the transplanted wild-type HSCs could be achieved at nonmyeloablative doses of CPA or IR in FAAKO mice also. Details of these results will be discussed.

**Gene Transfer by Receptor-Associated Protein / Polylysine Conjugates into HepG2 Cells.** *J.H. Kang<sup>1</sup>, T.G. Kim<sup>1</sup>, T.M. Yoo<sup>1</sup>, S.Y. Kang<sup>1</sup>, K.K. Jung<sup>1</sup>, M.Y. Cho<sup>1</sup>, J.I. Kim<sup>1</sup>, S.C. Jung<sup>2</sup>.* 1) Dept. of Pharmacology, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul, Korea; 2) Korea National Institute of Health, Seoul, Korea.

The 39-kDa receptor-associated protein (RAP) is a ligand for all members of the low density lipoprotein receptor family and is also able to be internalized into cells via receptor-mediated endocytic trafficking, which is an attractive mechanism for efficient gene delivery. In the present studies, we have developed a novel gene transfer agent using the RAP as a targeting ligand. The cDNA of partial RAP<sub>1-126</sub> (pRAP) except C-terminal heparin binding domain was amplified by PCR from human liver cDNA library and was re-amplified to add sequences of two cysteine residues at the carboxyl end to facilitate its conjugation to polylysine (PL), the DNA condensing carrier molecules. The RAP was purified at high yields using a bacterial expression system and coupled to polylysine via the heterobifunctional cross-linker SPDP. pRAP-PL conjugate, and PL as control, was condensed with a DNA expression plasmid containing the luciferase reporter gene driven by the CMV promoter under various salt conditions. Transfection into HepG2 cells by pRAP-PL/DNA complexes resulted in significantly higher expression levels in comparison to PL/DNA. Thus, this pRAP-PL conjugate may be useful as a nonviral gene delivery vector.

**Study of the Helper-Dependent Adenoviral Vector in the Delivery of a Therapeutic Intracellular Transgene Expressed in a Liver-specific Manner.** A. Mian, B. Mull, L. Pastore, G. Toietta, O. Bodamer, A. Beaudet, B. Lee. Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Developing liver-directed gene replacement therapy is an important alternative for treatment of cell autonomous enzymatic defects such as citrullinemia and other urea cycle disorders. These diseases require high-level gene expression and hepatocyte transduction. We have developed adenoviral vectors containing the human argininosuccinate synthetase (ASS) cDNA (mutant in citrullinemia) driven by ubiquitous and liver-specific promoters. In order to prolong transgene expression and decrease toxicity *in vivo*, we have employed a helper-dependent adenoviral vector (HDV). Transgene activity is evaluated in tissue culture using a  $^{14}\text{C}$ -labeling assay to follow the production of argininosuccinate from aspartate. XC cells lacking endogenous ASS were transduced with HDV expressing ASS from the BOS promoter. These cells expressed ASS at levels similar to controls infected with the first generation adenoviral vector (FGV) expressing ASS from the CAG promoter. *In vivo* evaluation of these vectors in C57BL/6 mice showed comparable vector-specific ASS mRNA levels between FGV- and HDV-treated mice at 3 days post-injection. The HDV-treated mice did not show evidence of liver dysfunction at day 3, in contrast to FGV-treated mice, which showed marked hepatitis. Expression of ASS in FGV-treated mice was lost at 8 weeks post-injection, but in HDV-treated mice expression persisted at 8 weeks, albeit at significantly lower levels compared to the day 3-time point. Persistence of the vector was confirmed by southern and FISH analyses. Persistence of vector DNA concomitant with reduction in ASS expression from the HDV may be explained by a cytotoxic T-lymphocyte response against ubiquitously expressed ASS. We found that ASS expressed from the liver-specific albumin promoter in a FGV persisted in C57BL/6 mice in contrast to ASS expressed from the ubiquitous CAG promoter. Hence, our data suggest that liver-specific expression of an intracellular transgene using HDV may confer high-level and long-term expression that is required for correction of hepatic enzymatic defects like the urea cycle disorders.

**Isolation and characterization of a side population from adult mouse skin.** *F. Montanaro*<sup>1,2</sup>, *J. Volinski*<sup>1,2</sup>, *L.M. Kunkel*<sup>1,2</sup>. 1) Genetics, Children's Hospital, Boston, MA; 2) Genetics, Harvard University, Boston, MA.

Staining of bone marrow and skeletal muscle cells with the DNA binding dye Hoechst 33342 has been used to identify by FACS a Side Population (SP) of cells with stem cell properties. These cells express markers such as SCA-1 and CD34 associated with a non-committed phenotype, and when injected into lethally irradiated mdx mice are able to reconstitute the bone marrow and participate in skeletal muscle regeneration. Using this same staining paradigm, we identified SP cells in the skin of adult mice. These cells express the same surface markers as SP cells isolated from skeletal muscle, and like muscle SP cells are negative for markers specific to hematopoietic stem cells, such as c-kit, CD45, and CD 43. Furthermore, some markers that are expressed by muscle and skin SP cells are not found on bone marrow SP cells. Experiments by Watt and colleagues have shown that neonatal dermal fibroblasts can fuse with skeletal muscle fibres in vivo. In a series of preliminary experiments aimed at determining the trans-differentiation potential of skin SP cells, congenic dystrophic female mice were intravenously injected with male-derived skin SP cells and the contribution of skin SP cells to skeletal muscle regeneration was examined by Y-chromosome fluorescence in situ hybridization. Skin is an attractive source of stem cells since it is readily accessible and allows the combination of gene and cell therapy approaches while minimising the risks of immune rejection.

**In vivo selection from a phage display library of peptides capable of targeting the retina.** *M. O'Reilly, S. Millington-Ward, P.F. Kenna, A. Stitt, D. Allman, P. Humphries, G.J. Farrar.* Ocular Genetics Unit, Smurfit Institute of Genetics, Trinity College, Dublin, Ireland.

A 12-mer peptide library displayed on the PIII coat protein of M13 was systemically injected into a mouse. Retinas were harvested and the extracted phage re-injected. Following four rounds of enrichment the phage inserts were sequenced. 50% of the sequences were identical. Another peptide was selected 18% of the time and a third peptide 11% of the time. All three most common peptides have a small consensus epitope. The efficiency of targeting the retina by these selected peptides was analysed by comparing their homing capacity with peptides which were not selected by the enrichment process. EM studies were carried out to localise the phage within the retina. These peptides may have potential for use as tags for selective delivery of drug/gene therapeutics to specific tissues.



**Mutation-independent ribozyme-based therapies for Retinitis Pigmentosa.** *B.G. O'Neill, M. O'Reilly, S. Millington-Ward, G. Tuohy, P.F. Kenna, P. Humphries, J. Farrar.* Genetics, Trinity College Dublin, Dublin, Ireland.

A significant hurdle essential to overcome for the successful development of gene therapies for dominantly inherited diseases is the immense intragenic heterogeneity commonly associated with such disorders. The large number of distinct mutations that give rise to various dominant diseases makes the development of tailor-made mutation-specific therapeutics not economically feasible. Dominantly inherited forms of Retinitis Pigmentosa (RP) which are a group of hereditary degenerative diseases of the retina are no exception. For example, over 100 different mutations in the rhodopsin gene can give rise to adRP. In 1997, we published 3 mutation-independent ribozyme based 'suppression and replacement' strategies<sup>1</sup> (termed GeneXchange) which circumvent the difficulties of genetic heterogeneity thus enabling one therapeutic, in theory, to treat all patients suffering from adRP regardless of the primary defect. In these studies, hammerhead ribozymes have been used to achieve suppression. A battery of mutation independent hammerhead ribozymes directed to human rhodopsin transcripts have been designed and analysed in vitro. Kinetic parameters were determined to establish the most efficient ribozymes in vitro. To facilitate further experimentation, stable COS-7 cell lines expressing human rhodopsin RNA were generated. Stable COS-7 cell lines were transiently transfected with rhodopsin-specific monomeric ribozymes (Rz10 and Rz40) and a connected-type multimeric ribozyme (RzMM). Preliminary analysis indicates that one ribozyme, Rz40, is highly efficient achieving up to 40% reduction in rhodopsin mRNA. In addition, a murine model simulating the human form of adRP expressing both the wild-type human rhodopsin transgene, the Pro23His human transgene on a mouse rho<sup>-/-</sup> background has been generated for ribozyme analysis. Transgenic animals and viruses expressing Rz40 and replacement constructs encoding wild-type rhodopsin protein but masked from ribozyme suppression have been developed and are currently being tested in vivo. 1. Millington-Ward & O'Neill et al. Strategies in vitro for gene therapies directed to dominant mutations. *Hum Mol Genet.* 1997;6:1415-1426. <sup>1</sup>.

**Antisense hammerhead ribozymes for the down-regulation of the COL1A1 gene in murine models of osteogenesis imperfecta.** *M.W. Kilpatrick<sup>1</sup>, I. Toudjarska<sup>1</sup>, J. Niu<sup>1</sup>, R.J. Wenstrup<sup>2</sup>, P. Tsipouras<sup>1</sup>.* 1) Dept Pediatrics, UConn Health Ctr, Farmington, CT; 2) Human Genetics, Children's Hospital Research Foundation, Cincinnati, OH.

Osteogenesis imperfecta (OI) is a systemic heritable disorder of connective tissue whose cardinal manifestation is bone fragility. OI is caused by a mutation in one of the genes for type I collagen. Studies on the molecular pathology of OI have identified two mechanisms of collagen type I defects. In chain exclusion, the mutant chain is not incorporated into the collagen triple helix, while in chain non-exclusion, it is. The dominant-negative effect of non-excluded mutations must be taken into account in strategies aimed at correcting the collagen defects in OI individuals. Antisense hammerhead ribozymes are small catalytic RNA molecules that can be targeted to any RNA molecule containing a putative cleavage site. This has led to their application to the down-regulation of a variety of gene products, and to the proposal that they might be applied to the selective elimination of mutant gene products particularly those that exert a dominant-negative effect. Hammerhead ribozymes targeted to mutant type 1 collagen are therefore potential therapeutic agents for OI. Ribozymes were designed to selectively target the mutant minigene transcript expressed in MC3T3-C3 cells, a murine calvarial osteoblast cell line stably expressing a human COL1A1 minigene deleted for exons 6-46. This minigene has been used to create a transgenic mouse model of OI. Active, and control inactive ribozymes were tested in vitro both on mutant and normal targets and on total RNA from MC3T3-C3 cells. Active ribozyme cleaved its target with high efficiency and specificity in both a time and dose dependent manner. Following transfection of a ribozyme expressing construct into MC3T3-C3 cells, intracellular ribozyme was detected along with a relative reduction of mutant transcript level. As the effect of the ribozyme on its target is highly dependent on the ribozyme to target ration, multimeric ribozyme cassettes have been constructed and are being tested for efficacy in cell culture prior to their application in transgenic OI models.

**Delivery of Functional Four-Repeat Micro-Dystrophin to *Mdx* Muscle Via Different AAV Serotypes.** *S. Harper*<sup>1</sup>, *C. DelloRusso*<sup>1</sup>, *R. Crawford*<sup>1</sup>, *H. Harper*<sup>1</sup>, *J. Engelhardt*<sup>2</sup>, *D. Duan*<sup>2</sup>, *J. Chamberlain*<sup>1</sup>. 1) Dept of Neurology, University of Washington, Seattle, WA; 2) Dept of Anatomy and Cell Biology, University of Iowa, Iowa City, IA.

The goal of this study was to evaluate the correction of Duchenne muscular dystrophy (DMD) in the *mdx* mouse model using different serotypes of AAV carrying extremely small dystrophin genes (~3.5 kb vs. the full-length 14 kb). The rod domain of dystrophin is composed of 24 spectrin-like repeats, and natural, in-frame deletions of this region lead to a milder form of dystrophy called Becker MD. Based on our prior analysis in transgenic mice, we created several rod domain and C terminal deletions that resulted in dystrophin cDNAs small enough to fit into AAV. AAV is a highly efficient vector that does not elicit an immune response in dystrophic muscle if it is carrying a gene driven by a muscle-specific promoter. AAV-2 is the most prevalent and well-characterized system, but recent studies show that serotypes 1, 5, and 6 boost gene expression 100- to 1000-fold in mouse muscle. We generated transgenic mice expressing several different micro-dystrophin clones containing only four repeats, and analyzed their ability to correct muscular dystrophy by both morphological and physiological assays. Two of three transgenes significantly reduced the amount of dystrophy observed in the *mdx* limb and diaphragm muscles. The best transgene (DR4-R23) produced muscle with wild-type levels of central nuclei, membrane integrity, resistance to contraction-induced injury, and the ability to run on a treadmill. No areas of fibrosis nor monocyte infiltration were observed. We subsequently demonstrated that highly functional micro-dystrophins can be successfully delivered via AAV-2 to muscles of young adult *mdx* mice. We are currently testing gene expression of our best micro-dystrophin with alternate AAV serotypes, a more efficient Kozak sequence, and a highly active, mutant form of the muscle creatine kinase promoter (CK6). Functional correction of muscular dystrophy using these vectors will be compared to results obtained with our gutted adenovirus vector system that expresses full-length dystrophin.

**Gene Transfer with VSV-G/Plasmid Complexes and with Ternary Complexes of VSV-G, plasmid and Envelope Protein-free Retrovirus-like Particles.** *A. Miyano-hara, T. Friedmann.* Pediatrics, UCSD School of Medicine, La Jolla, CA.

We have previously demonstrated that the G protein of vesicular stomatitis virus (VSV-G) forms complexes with liposomes to produce stabilized particles with enhanced transfection capability. We have also demonstrated earlier that VSV-G can convert non-infectious, envelope protein-free virus-like ("bald") particles to infectious form in cell-free conditions in vitro. We demonstrate now that VSV-G interacts with plasmid DNA alone to produce complexes with 100-1000 fold increased transfection properties in several cell types compared with plasmid alone. Interaction of VSV-G and plasmid with non-infectious bald particles produces ternary complexes that display gene transfer properties. The polycation polybrene is required for the formation of these transfection-competent complexes. We interpret these results to indicate that VSV-G is able to provide a structural as well as a fusiogenic function to complexes with naked DNA and to immature virus-like bald particles, that such complexes are thereby able to enter target cells and display enhanced gene transfer properties. We suggest that these techniques can help elucidate mechanisms of virus assembly and aid in the development of a variety of novel gene transfer methods. These studies were supported by grant HL64730 from the National Heart, Lung and Blood Institute.

**Long term correction of lipid storage in multiple organs of Fabry mice by direct injection of AAV vectors into skeletal muscle.** *T. Shimada<sup>1</sup>, H. Takahashi<sup>1</sup>, Y. Hirai<sup>1</sup>, K. Takahashi<sup>1</sup>, M. Migita<sup>1</sup>, H. Sakuraba<sup>2</sup>, R. Kase<sup>2</sup>, Y. Hashimoto<sup>3</sup>.* 1) Biochem. Mol. Biol., Nippon Medical School, Tokyo, Japan; 2) The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; 3) The Institute of Physical and Chemical Research, Saitama, Tokyo, Japan.

Fabry disease is an X-linked inherited metabolic disorder due to a deficiency of the lysosomal enzyme,  $\alpha$ -galactosidase A ( $\alpha$ -gal A). The enzyme defect leads to the systemic accumulation of neutral glycosphingolipids predominantly ceramide trihexoside (Gb3). Clinical trials of enzyme replacement therapy are now being conducted. As an alternative approach, we are studying the feasibility of adeno-associated virus (AAV) vector mediated gene therapy. We injected the AAV vector containing the  $\alpha$ -gal A gene driven by the CAG promoter ( $1.5 \times 10^{11}$  particles) into the right quadriceps muscles of Fabry knock-out mice (12 weeks old). A time course study showed that  $\alpha$ -gal A activity in plasma was increased to approximately 20 % of the normal mice that persisted for up to at least 38 weeks. In parallel with the plasma concentration, the  $\alpha$ -gal A activities in multiple organs including liver, heart, and kidney were continued to be 5-15 % of those observed in the normal mice. Finally, quantitative measurement of glycolipids showed that the concentrations of Gb3 decreased to normal levels in various organs including kidney at 38 weeks. The reduced Gb3 level was also confirmed by immunohistochemical analysis using the anti-Gb3 specific antibody. These results indicate that  $\alpha$ -gal A secreted from transduced muscle cells entered into affected tissues and efficiently cleared lipid. The safety of direct injection of AAV vectors into skeletal muscle has been well established. AAV mediated muscle directed gene therapy should be a clinically relevant therapeutic approach for Fabry and other lysosomal disorders.

**Amelioration of dystrophic phenotypes in transgenic *mdx* mice expressing truncated dystrophin cDNA.** *M. Sakamoto, K. Yuasa, T. Yokota, S. Masuda, Y. Miyagoe-Suzuki, S. Takeda.* Molecular Therapy, Natl Inst Neurosci NCNP, Kodaira, Tokyo, Japan.

Duchenne Muscular Dystrophy (DMD) is an X-linked, lethal disorder caused by a defect in the *dystrophin* gene. Progressive muscle weakness, cardiomyopathy and early death characterize the disease. Dystrophin is localized at the inner surface of the plasma membrane and forms the complex with dystrophin-associated proteins (DAPs) to stabilize the sarcolemma by linking the cytoskeleton to the extracellular matrix. Dystrophin has a central rod domain, which consists of 24 triple-helical repeats and 4 hinge segments, and accounts for 76% of the molecule. It has been shown that a large in-frame deletion in this domain results in a mild allelic form of the disease, Becker muscular dystrophy (BMD).

Adeno-associated virus (AAV) vector-mediated micro-dystrophin cDNA transfer is one of attractive approaches for the treatment of DMD. AAV vector allows long-term expression of the transferred gene without significant immune responses, while it has a limited insertion size up to 4.7-4.9 kb. Therefore, we cannot accommodate a full-length dystrophin cDNA (14 kb) into AAV vector. To find a functional, but small-sized dystrophin, we generated a series of rod-truncated micro-dystrophin cDNAs with one rod repeat and two hinges (named M3 construct), three rod repeats and two hinges (AX11 construct), and four rod repeats and three hinges (CS1 construct). We have generated transgenic *mdx* mice expressing micro-dystrophins and evaluated their function to improve dystrophic phenotype. Histological examination showed that CS1 construct recovered DAPs at the sarcolemma and significantly reduced muscle degeneration in the hind limb muscles and diaphragm. On the other hand, M3 construct could not ameliorate *mdx* phenotype in spite of recovery of DAPs at the sarcolemma. These data suggest that the rod structure, especially its length is critical for function of micro-dystrophin. We are now investigating the phenotypes of AX11-transgenic *mdx* mice. Our goal is to identify a functional micro-dystrophin and transfer it into dystrophin-deficient dystrophic muscles using AAV vector.

**Inhibition of caspase activity and prevention of apoptosis in 661W cone photoreceptor cells.** *G. Tuohy, P.F. Kenna, P. Humphries, G.J. Farrar.* Genetics, Trinity College Dublin, Dublin, Ireland.

Retinitis pigmentosa (RP) is an hereditary group of degenerative retinopathies with an incidence world-wide of approximately 1 in 4000. Over 30 distinct genes have been implicated in the etiology of RP to date however, despite such genetic heterogeneity, apoptosis appears to be a final common pathway of photoreceptor cell death in these disorders. RP may be additionally characterised by its primary effect on the rod cell population of the retina with patients initially reporting a narrowing of the visual field and loss of scotopic vision. Rod cell loss is generally followed by a further wave of apoptotic cell death in the cone cell population eventually leading to loss of photopic vision. Extending the longevity of cone cells in the retina, despite rod photoreceptor cell loss, represents a potentially valuable therapeutic goal in limiting a number of the more severe medical consequences of RP. 661W cone cells represent a photoreceptor transformed cell line originally derived from a transgenic mouse expressing the SV-40 T antigen in the retina. 661W cells have been shown to express opsin, arrestin, phosphodiesterase (PDE),  $\gamma$ -transducin and rds/peripherin. In this study cultures of 661W cells were exposed to a variety of apoptotic stimuli including UV irradiation and chemotherapeutic agents. Cultures pre-treated with as little as 80  $\mu$ M Z.VAD.FMK and 400ng baculoviral p35 showed significant resistance to apoptotic cell death following induction by UV and daunorubicin. Survival rates for 661W cells treated with 80  $\mu$ M Z.VAD.FMK and 400ng baculoviral p35 were 72.360.93% and 64.151.80% respectively compared to controls where survival was 6.991.35% and 17.491.55% for Z.VAD.FMK and p35 respectively. Therapeutic strategies directed at modulating caspase activity may thus present a viable means of blocking apoptotic cone cell death in the context of degenerative retinopathies.

**Gene therapy for Fabry disease.** *R. Ziegler<sup>1</sup>, N. Yew<sup>1</sup>, C. Li<sup>1</sup>, M. Cherry<sup>1</sup>, M. Przybylska<sup>1</sup>, D. Armentano<sup>1</sup>, R.J. Gregory<sup>1</sup>, S.C. Wadsworth<sup>1</sup>, R.J. Desnick<sup>2</sup>, Y.A. Ioannou<sup>2</sup>, S.H. Cheng<sup>1</sup>.* 1) Genzyme Corp, Framingham, MA; 2) Mt. Sinai Sch. of Med. New York, NY.

Fabry disease is a monogenic X-linked disorder caused by a deficiency of the lysosomal enzyme  $\alpha$ -galactosidase A. Clinical manifestations are due to progressive accumulation of the substrate globotriaosylceramide (GL-3) in the vascular endothelium of heart, kidney, skin, and nervous system. Feasibility of gene therapy for treating Fabry disease was established using systemic delivery of a recombinant adenoviral vector encoding  $\alpha$ -galactosidase A into Fabry mice. This resulted in correction of both the enzymatic and lysosomal storage defects in all affected organs. However, there was significant liver toxicity associated with administration of this vector, and transgene expression was transient. We are currently developing alternative vectors with improved safety and expression profiles such as pseudo-adenoviral vectors, adeno-associated viral vectors as well as synthetic vectors. Systemic administration of  $5 \times 10^{11}$  particles of AAV/CMVHIgal into immunosuppressed Fabry mice resulted in expression of approximately 10% normal levels of  $\alpha$ -galactosidase A in the liver and significant reductions in GL-3 levels in most organs. By replacing the CMV promoter with a liver-specific promoter (albumin) we obtained levels of  $\alpha$ -galactosidase A that were within the normal range in livers of immune competent Fabry mice. In contrast to studies using the CMV promoter, only very low titers of anti- $\alpha$ -galactosidase A antibodies could be measured when the enzyme was transcribed using this liver-specific promoter. We have also developed improved synthetic vectors. Significantly higher and more persistent expression has been obtained by optimizing transcription cassettes, and by reducing the inflammatory characteristics associated with CpG motifs on the pDNA. After IN administration of the optimized vector,  $\alpha$ -galactosidase A expression from pGZCUBI-HAGA (containing a hybrid CMV enhancer-ubiquitin promoter) increased over time and by day 35 was 10- to 15-fold higher than starting levels. These two improved vector platforms show promise for a safe, efficacious gene therapy treatment for Fabry disease.



**Partial correction of murine hemophilia A by murine factor VIII cDNA delivered via AAV vector.** *R. Sarkar<sup>1</sup>, W. Xiao<sup>2</sup>, H.H. Kazazian<sup>1</sup>*. 1) Dept Genetics, Univ Pennsylvania Sch Medicine, Philadelphia, PA; 2) Division Of Hematology, Children's Hospital of Philadelphia, Medicine, Philadelphia, PA.

Hemophilia A is an X linked clotting recessive disorder that affects 1 in 10,000 males. Earlier, using first generation adenoviral vectors, we reported partial long-term correction of murine hemophilia A with murine FVIII. Despite phenotypic correction, the immune system still recognized a species-specific transgene protein as a neo-antigen, eliciting a cytotoxic T cell response. Recombinant AAV vectors are known to maintain long-term gene expression without eliciting destructive cell-mediated immune responses. These features in association with AAV's lack of pathogenicity and broad tissue tropism make it an attractive vector for human gene therapy. Unlike the successful development of AAV vectors for gene delivery in hemophilia B, applications to hemophilia A have been limited by the size constraint of the parvovirus (5kb). In this study we designed an AAV vector system that could successfully deliver FVIII cDNA and potentially correct the bleeding phenotype in hemophilic mice. The AAV-FVIII cassette contains a short basic albumin promoter (0.2kb), a B domain-deleted murine cDNA with an intron (4.4kb), and a short poly A signal (0.1kb). The entire cassette is flanked by ITRs of 145bp each. FVIII knockout mice were injected with  $1 \times 10^{11}$  viral particles/mouse via the spleen. The mice were bled initially at 2 weeks, and followed at monthly intervals up to six months post injection of either the FVIII cDNA virus alone or the FVIII cDNA virus in combination with a second virus bearing an albumin enhancer. Of 24 mice, 14 mice survived the tail clip at 3 months with FVIII activities of 2.5 to 3 percent. At six months these mice continued to express FVIII activity at levels 2.5 to 3 percent. 10 mice with 0 to 2.5 percent FVIII activities at 3 months did not survive tail clip. No differences were observed between the mice receiving FVIII cDNA alone and those receiving both FVIII and the enhancer. Further studies are ongoing on these mice and others given FVIII via portal vein and tail injection.

**TREATMENT OF THE MOUSE MODEL OF MPSI BY TRANSPLANTATION OF BONE MARROW AND OF GENETICALLY MODIFIED BONE MARROW.** *Y. Zheng*<sup>1</sup>, *J. Zhou*<sup>1</sup>, *R. Rozengurt*<sup>2</sup>, *S. Ryazantsev*<sup>1</sup>, *H.H. Li*<sup>1</sup>, *D.B. Kohn*<sup>3</sup>, *E.F. Neufeld*<sup>1</sup>. 1) Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA; 2) Dept. of Pathology & Laboratory Medicine, UCLA School of Medicine, Los Angeles, CA; 3) Dept. of Molecular Microbiology and Immunology, Childrens Hospital Los Angeles, Los Angeles, CA.

Mucopolysaccharidosis I (MPS I,  $\alpha$ -L-iduronidase deficiency) includes the Hurler syndrome and the less severe forms, Hurler-Scheie and Scheie syndromes. Bone marrow transplantation is known to alter the course of the disease in Hurler patients, and direct administration of recombinant  $\alpha$ -L-iduronidase (Idua) to MPS I patients is currently undergoing clinical trial. To test the potential of gene therapy, we developed an MPS I mouse model by homologous recombination, inserting *neo*<sup>r</sup> in place of exon 6, as had been previously done by Clarke et al (Human Mol Genet 6:503, 1997). The mutant *Idua* was placed on a C57BL/6 background. Bone marrow transplantation with wild-type or mutant bone marrow was performed when the *Idua* <sup>-/-</sup> mice were 6-8 weeks old. For genetic modification, the bone marrow was transduced with human *IDUA* cDNA in an MND retroviral vector or sham-transduced with the same vector carrying *EGFP*. Recipient mice were sacrificed 2 and 6 months later for analyses. The liver and spleen of animals receiving wild type bone marrow had a level of enzyme that was considerably below normal, but sufficient to reduce to normal the accumulation of soluble glycosaminoglycans (GAG) and the secondary elevation of b-hexosaminidase. Only a marginal amount of Idua appeared in the kidney and none at all in the brain. On the other hand, when the bone marrow was transduced with *IDUA*, not only did the liver and spleen contain many times the normal level of Idua, but so did the kidney, with concomitant normalization of GAG. Even in the brain, the level of Idua was normal or higher in the three mice analyzed. The morphology of the liver, examined by EM, had returned to normal, but the effect of the enzyme on the pathology of the brain has not yet been determined. The results encourage us to think that optimization of the procedure may permit vector-containing cells to reach many tissues. (Supported in part by NIH grant DK 38857).

**Adeno-associated virus vector-mediated gene transfer into anterior horn cells.** *G. Acsadi<sup>1,2</sup>, H. Yang<sup>1</sup>, R. Anguelov<sup>1</sup>, G. Toht<sup>1</sup>, Y. Wang<sup>1</sup>, M.E. Shy<sup>2</sup>*. 1) Dept. Pediatrics, Wayne State Univ, Detroit, MI; 2) Dept. of Neurology, Wayne State Univ., Detroit, MI.

In gene therapy for motor neuron diseases, such as spinal muscular atrophy and amyotrophic lateral sclerosis, the target cells for gene transfer are skeletal muscle cells and neurons. Previously, we showed that recombinant adenoviruses (ADV) are efficient vectors for gene transfer into muscle, and (by retrograde transport) into motor neurons of neonatal mice, but with a lower efficiency in adult mice. AAV is known to transduce both immature and mature muscles without causing a significant immunoreaction. However, the ability of AAV to transfer genes into lower motor neurons has not been investigated yet for gene therapy purposes. The objective of this study was to determine whether AAV could be efficiently used to transfer foreign genes into spinal cord motor neurons. We have constructed AAV with CMV-EGFP (green fluorescent protein) and GDNF expression cassettes, using a helper virus free system. The AAV was grown and purified to high titer and injected into hindlimb muscles of neonatal and adult mice. EGFP expression was analyzed from muscle and spinal cord by a fluorescent microscope and GDNF expression was analyzed by chemiluminescent ELISA and real-time PCR. EGFP and GDNF expression was detected in both neonatal and adult muscles three weeks after vector administration. Furthermore, EGFP was detected in large number of spinal cord motor neurons at both ages, suggesting that a retrograde transport of transgene product and/or AAV genome has occurred after intramuscular administration. We conclude that AAV, similarly to ADV, can be used to introduce transgenes into muscle cells and motor neurons. Since AAV causes less of an inflammatory response than ADV, it may have an advantage over ADV in developing effective gene therapy for motor neuron diseases. Supported by MDA.

**Generation of Transplantable Cells for Ex vivo Gene Therapy of Globoid Cell Leukodystrophy (Krabbe disease).**

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Globoid cell leukodystrophy (GLD) or Krabbe disease is a disease of humans and several animal species caused by the deficiency of galactocerebrosidase (GALC) activity. This deficiency results in the accumulation of a cytotoxic substrate called psychosine and a severe decrease in central and peripheral nervous system myelin. At this time treatment in humans is limited to bone marrow transplantation. The use of stem cells that have been transduced to produce high GALC activity might be ideal for treating GLD. In order to generate transplantable cells with high GALC expression that can be traced through a reporter gene, we initially prepared several retroviral constructs containing a combination of human (h) or murine (m) GALC cDNA, internal ribosomal entry site (IRES), green fluorescent protein (GFP) and puromycin resistant gene (Puro) sequences. Each time an extra element was added to the viral construct, considerable decline in GALC activity was observed. By generating two MFG-based viral vectors to co-infect the target cells, followed by puromycin selection, we have developed a method for obtaining cells that maintain high GALC expression while they also express GFP and are puromycin resistant. We have placed both human and mouse GALC cDNAs in the MFG vector, which lacks a selectable marker. Amphotropic viral particles were produced after transfection into psi-CRIP cells. Using these viral particles, we have previously shown that infectious virus can successfully transduce many deficient cell types including astrocytes and oligodendrocytes resulting in high GALC activity and normal phenotype. In a separate experiment, psi-CRIP cells were transfected with MFG vector containing GFP-IRES-Puro, and viral producing colonies were selected with puromycin. Subsequent co-infection of dividing cells with both viral vectors followed by selection with puromycin resulted in cells expressing GFP alone or GFP and exogenous GALC. Colonies expressing high GALC were selected by enzyme assay and expanded for injection into the mouse models of GLD.

**Autoimmune scurfy mice, the ortholog of human IPEX, are rescued by transplantation of bone marrow or T cells.** R.S. Wildin, S. Smyk-Pearson. Molecular & Medical Genetics, Oregon Health & Science Univ, Portland, OR, USA.

**PURPOSE:** We assessed the therapeutic value of bone marrow or peripheral T cell transplantation for treating the fatal autoimmune disorder in the *sf* mouse. **BACKGROUND:** The syndrome of Immunodysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) is a T cell-mediated autoimmunity disorder due to mutation of *FOXP3*. IPEX is generally fatal in infancy. One patient responded to a bone-marrow transplant despite limited engraftment. Thus, normal cells may suppress the antigen hyper-reactivity of *FOXP3*-deficient T cells. The scurfy (*sf*) mouse is a natural mouse model for IPEX and shares clinical features including lymphadenopathy and eczema. Untreated *sf* mice die at 21 - 28 days. **METHODS:** We injected  $5 \times 10^6$  syngeneic normal bone marrow cells IP into 5 newborn *sf/Y* mice after 5 Gy (sublethal) irradiation (BMT). We injected  $2 \times 10^7$  syngeneic normal T cells into 6 additional *sf/Y* mice at 12 days of age without conditioning (TCT). We observed treated and control mice for disease features and lifespan. **RESULTS:** Most BMT and TCT mice developed scurfy skin features in a delayed fashion. BMT mouse 1 had little skin disease but died at 30 days. The absence of spleen, lymph nodes and thymus suggested that death was due to engraftment failure rather than scurfy disease. BMT mouse 2 was euthanized at 64 days due to severe tail eczema, but lymph nodes, thymus, and spleen appeared normal, suggesting that *sf*-like lymphoproliferation had been eliminated. BMT mouse 3 died at 47 days. BMT mice 4 and 5 currently survive (76 and 22 days) with eczema but are behaving normally and the skin of the older mouse is improving. Three TCT mice lived 5 days longer than PBS-injected controls. The other TCT mice currently survive at 75, 52, and 28 days. The eldest appears nearly normal. **CONCLUSIONS:** Bone marrow transplantation in the newborn scurfy mouse can slow disease and prevent death. BMT may prove generally useful in treating IPEX patients. T cell transplantation in scurfy mice may prolong life. Normal T cells can suppress the abnormal reactivity of *Foxp3*-deficient T cells *in vivo*, raising the possibility of alternative treatments for IPEX patients.

**A double blind therapeutic trial in Angelman syndrome using betaine and folic acid.** CA. Bacino, J. Goddard-Finegold, M. Turcich, D. Glaze, W. O'Brien, AL. Beaudet. Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Angelman Syndrome (AS) is caused by maternal deficiency of the UBE3A; this gene encodes E6-AP ubiquitin-protein ligase and is subject to genomic imprinting with paternal silencing in the brain. It is known that expression of UBE3A from the maternal copy of chromosome 15q11-q13 is essential to prevent AS. In 1998 a patient with methylene-tetrahydrofolate reductase (MTHFR) deficiency was described with clinical features indistinguishable from AS. This could suggest that abnormalities in the folic acid pathway might result in abnormalities of DNA methylation that affect expression of UBE3A causing genocopies of AS. The rationale of this protocol is based on activating the silent paternal allele for UBE3A in AS patients, perhaps by silencing the antisense transcript in this region and increasing the "leakiness" of the paternal allele. A total of 20 patients with AS are currently being enrolled in a randomized, double blind protocol using 6 or 12 gm/d of betaine and 15 mg/d folic acid versus placebo. The plan is to continue treatment for one year with careful evaluation of neurologic, nutritional, and developmental status, including language, motor, and adaptive domains. The evaluation includes a neurologic exam and EEG. Gait, station, abnormal movements, and progression of neurologic signs over time are assessed. The Revised Bayley Scales of Infant Development are used to provide psychomotor and mental developmental indices. We are also using the Vineland Inventory to assess behavioral and emotional development Blood studies include, CBC, BUN, methylation studies of DNA, and levels of red cell folate, homocysteine, methionine and betaine. Thus far, 12 children with AS have been enrolled. Initial results and protocol rationale will be discussed. The strategy of activating silenced alleles for treatment of genetic deficiency of imprinted genes could be considered in other disorders such as Prader-Willi syndrome.

**Improvement in sterol levels with cholesterol therapy in Smith-Lemli-Opitz syndrome (SLOS).** *M. Irons*<sup>1</sup>, *E.R. Elias*<sup>2</sup>, *C. Bay*<sup>3</sup>, *B. Pober*<sup>4</sup>, *G.S. Tint*<sup>5</sup>, *G. Salen*<sup>5</sup>. 1) Children's Hospital, Boston, MA; 2) Children's Hospital, Denver, CO; 3) Children's Hospital, Pittsburgh, PA; 4) Yale-New Haven Medical Center, New Haven, CT; 5) VA Medical Center, East Orange, NJ.

SLOS is an autosomal recessive disorder of cholesterol biosynthesis characterized by decreased levels of cholesterol and increased levels of the cholesterol precursors, 7-dehydrocholesterol (7-DHC) and 8-dehydrocholesterol (8-DHC). Treatment with supplemental cholesterol was initiated to determine if such treatment could improve sterol levels and ultimately the clinical problems seen in affected patients. Seventeen patients (11 females/6 males, currently ages 1-20 years) have been treated with a suspension of crystalline cholesterol in oil (50-300 mg/kg/day). Initial cholesterol levels range from 8-154 mg/dl. Ages at initiation of therapy range from birth to 15 years, and durations of therapy range from 3 months to 7.5 years. Patients were divided into mild (7), moderate (5), and severe (5) groups based on pretreatment cholesterol levels and severity of clinical presentation. Levels of cholesterol and the ratio of cholesterol to total sterols increased significantly for all patients combined ( $p < 0.0001$  for each), as well as within each clinical severity subgroup ( $p < 0.05$ ). 7-DHC ( $p = 0.009$ ) and 8-DHC ( $p = 0.003$ ) levels decreased significantly for all patients combined as well as for patients in the moderate group. Significant decreases in 7-DHC ( $p = 0.014$ ) and 8-DHC ( $p = 0.006$ ) were noted when combining the moderate and severe groups. Overall, 7-DHC and 8-DHC were reduced by 36% and 44% respectively, while cholesterol and the ratio of cholesterol to total sterols increased by 61% and 27%, respectively. Clinical response was variable. No toxicity has been noted in over 7 years of therapy. In summary, treatment of patients with SLOS with a suspension of crystalline cholesterol in oil results in improvement in sterol levels without long-term toxicity.

**Endothelial dysfunction in patients with MELAS: role of oxidative stress to stroke.** *Y. Koga<sup>1</sup>, H. Matsuoka<sup>2</sup>, R. Fukiyama<sup>1</sup>, I. Ueki<sup>1</sup>, Y. Akita<sup>1</sup>, T. Imaizumi<sup>2</sup>.* 1) Dept Pediatrics & Child Health, Kurume Univ Sch Medicine, Fukuoka, Japan; 2) Department of 3rd Internal Medicine, Kurume University School of Medicine, Fukuoka, Japan.

Mitochondrial angiopathy demonstrating degenerative change with increased abnormal mitochondria in the endothelial cells of intramuscular small arteries and arterioles have been reported in many MELAS patients. However, the primary cause of the young MELAS strokelike episodes is still controversial. Since abnormal mitochondria generates superoxide anion, we hypothesized that vascular complications in MELAS may be associated with endothelial dysfunction caused by oxidative stress. Six patients have an A3243G mutation, and one patient has a T3271C mutation in the mitochondrial tRNA<sup>Leu</sup>(UUR) gene. In this study, we examined flow-mediated vasodilatation, as a non-invasive measure of endothelial function, and effects of an antioxidant, vitamin C in patients with MELAS. We also studied the pharmacological effect on the clinical course, and biochemical parameters after administration of L-arginine to a patient in the acute phase of stroke on three separated occasions and, and on the functional aspects of the cerebral hemodynamics using SPECT. Flow-mediated vasodilatation was significantly less (10% of the age-matched controls) in MELAS patients. Endothelium-dependent vasodilatation induced by glyceryl trinitrate was also impaired. Vitamin C administration significantly restored flow-mediated dilation and glyceryl trinitrate-induced vasodilatation to near-normal levels in MELAS but did not affect them in controls. After the administration of L-arginine, all the symptoms were clinically improved, and the intracranial hemodynamics were also improved in the ischemic area (in the left temporal lobe), but unchanged in the brain stem (thalamus). Our data demonstrated that angiopathy seen in MELAS involved abnormality in the capacity of vasodilatation in the endothelial system, which may play an important role in causing strokelike episodes in this disorder.



**Toward Repair of Retinal Degenerations with Differentiated and Embryonic Stem (ES) Cells.** *S.H. Tsang<sup>1,2</sup>, D.S. Salchow<sup>2</sup>, M. Jiang<sup>2</sup>, J. Kong<sup>2</sup>, M. Redmond<sup>3</sup>, S.P. Goff<sup>2</sup>, P. Gouras<sup>2</sup>, D.B. Farber<sup>1</sup>.* 1) UCLA, Los Angeles, CA; 2) Columbia U, NY, NY; 3) NIH, Bethesda, MD.

Leber Congenital Amaurosis (LCA) is a recessive dystrophy resulting from defects in either the retinal pigment epithelial (RPE) or photoreceptors. We are studying two mouse models of LCA: *Rpe65<sup>TM</sup>/Rpe65<sup>TM</sup>* mice carry a null mutation in *Rpe65* (Redmond et al., Nat Genet 20, 344) and, *Pdeg<sup>TM</sup>/Pdeg<sup>TM</sup>* mice (Tsang et al, Science, 272, 1026) carry a targeted disruption in *Pdeg*, the gene encoding the g subunit of the cGMP phosphodiesterase (PDEg). Both animal models have ERG features seen in individuals with LCA. We are testing whether transplantation of RPE cells into *Rpe65<sup>TM</sup>/Rpe65<sup>TM</sup>* mice, and ES cells into *Pdeg<sup>TM</sup>/Pdeg<sup>TM</sup>* mice have therapeutic effects. In the latter case, we are probing if ES cells can differentiate into photoreceptors after transplantation in *Pdeg<sup>TM</sup>/Pdeg<sup>TM</sup>* mice. A GFP cassette has been placed under the control of the opsin gene in ES cells. This GFP reporter will only be active if the ES cells differentiate into rod photoreceptors and its expression can be followed using a scanning laser ophthalmoscope (SLO). Following subretinal injection of wild-type RPE or ES cells ( $5-10 \times 10^4$  cells) expressing the green fluorescent protein (GFP) with the polymerase II or the opsin promoter into one eye, dark-adapted corneal ERGs of both eyes were performed at different ages. In the *Rpe65<sup>TM</sup>/Rpe65<sup>TM</sup>* model, RPE transplantation increased ERG responses. Although transplanted ES cells were found in the *Pdeg<sup>TM</sup>/Pdeg<sup>TM</sup>* mice, preliminary studies suggest naive ES cells fail to express sufficient levels of GFP detectable by SLO. Teratocarcinomas frequently associated with ES cell transplantation have not been observed in our animals. Transplanted RPE cells can slow degeneration in *Rpe65<sup>TM</sup>/Rpe65<sup>TM</sup>* mice. Naive ES cells are able to survive in the subretinal space of *Pdeg<sup>TM</sup>/Pdeg<sup>TM</sup>* mice. We are now testing transplanted ES cell-derived embryoid bodies for photoreceptor differentiation. Transplantation of RPE and/or differentiated ES cells could become a therapy for selected individuals with LCA and associated retinal dystrophies.

**Modulation of muscular dystrophy by inhibitors of apoptosis.** *S. Abmayr, R.W. Crawford, J.S. Chamberlain.* Dept Neurology, Univ Washington, Seattle, WA.

Duchenne muscular dystrophy (DMD) is a human X-linked recessive disorder caused by mutations in the dystrophin gene. DMD is characterized by a progressive loss of muscle function, leading to death in the early twenties. The relationship between the protein deficiency and the late clinical onset of muscle pathology remains unclear. Several lines of evidence support the hypothesis that cell death of dystrophin deficient muscle might be initiated by apoptosis, followed by necrotic processes. We have been studying the role of proteins that repress apoptosis or/and enhance muscle regeneration for their ability to modulate the dystrophic phenotype in conjunction with gene replacement therapy. We have generated transgenic mice that overexpress ARC, an apoptosis inhibitor, almost exclusively expressed in skeletal muscle and heart. ARC was shown previously to interact selectively with caspases and to prevent hypoxia-induced release of cytochrome c from mitochondria. Morphology studies were performed to determine the level of de- and regeneration of muscle fibers of ARC transgenic/*mdx* mice in comparison with *mdx* controls. Tg/*mdx* mice displayed a clear pattern of *mdx* pathology. We performed immunohistochemistry analysis to compare the level of activated caspase-3 in *mdx* and transgenic/*mdx* muscle and detected no significant difference in the levels of activation. Caspase-3 positive fibers displayed membrane damage as assessed by uptake of the vital dye Evans blue, suggesting apoptotic pathways may be activated as a result of membrane leakage. Together the results suggest that overexpression of ARC does not overtly protect *mdx* muscle from dystrophic pathology. Currently we are investigating if ARC plays an inhibitory role in hypoxia-induced apoptosis in muscle, which might contribute to improved muscle function in less sedentary animals. In a complementary approach, we are examining the role of IGF-I in muscle and its ability to modulate the *mdx* muscle pathology. IGF-I has been shown to enhance muscle regeneration and to prevent age-related declines in muscle mass and function. We have generated adenoviral vectors expressing IGF-I and dystrophin and are studying their effects in preventing and reversing dystrophic pathology in *mdx* mice.

**Response to olanzapine influenced by genes in neurotransmitter receptors.** *M.J. Arranz<sup>1</sup>, S. Staddon<sup>1</sup>, D. Mancama<sup>1</sup>, J. Munro<sup>1</sup>, S. Osborne<sup>1</sup>, M. Beperet<sup>2</sup>, I. Mata<sup>2</sup>, R. Kerwin<sup>1</sup>.* 1) Dept. Psychological Medicine, Institute of Psychiatry, London, England; 2) Fundacion Argibide, Instituto de Salud Mental de Navarra, Pamplona, Spain.

In the last decade pharmacogenetic studies have shown that response to drug treatment can be influenced by genetic variants in target sites. Several studies have shown that polymorphisms in dopaminergic and serotonergic receptors can influence general response to antipsychotics (Arranz et al., 1998; Wong et al., 2000) and development of side effects (Basile et al., 2000). In a previous study we also reported that response to the potent antipsychotic clozapine could be predicted by a combination of polymorphisms in key receptor genes (Arranz et al., 2000). In the present study we report the investigation of the relation of polymorphisms in serotonergic and dopaminergic receptors and response to the atypical antipsychotic olanzapine. A group of 100 patients treated with olanzapine were genotyped for polymorphisms in HTR2A, HTR2C, HTR6, hSERT and DRD3 genes. Genotype and allele distributions between olanzapine responders and non-responders resembled those described for clozapine response. Strong associations were observed between a DRD3 Ser9Gly polymorphism and improvement of positive symptoms particularly in a subgroup of patients of basque origin ( $p < 0.01$ ). In addition, combination of these information resulted in the prediction of olanzapine response with more than 80% success ( $p = 0.04$ ). We are expanding these studies to other antipsychotic drugs in the hope of being able to genetically select the most beneficial drug for each individual even before starting the treatment.

**Aclarubicin stimulates production of full length SMN from the SMN2 gene and restores normal SMN protein levels to cells from type I SMA patients.** *C. Andreassi, D.D. Coover, U.R. Monani, A.H.M. Burghes.* Dept. of Molec. and Cell. Biochemistry and Dept. of Neurology, College of Medicine and Dept. of Molecular Genetics, College of Biological Sciences, The Ohio State University, Columbus, OH.

Proximal spinal muscular atrophy (SMA) is a common autosomal recessive disorder characterized by degeneration of a-motor neurons in the spinal cord. SMA is caused by mutation of the telomeric survival of motor neuron gene SMN1 but not mutation of the centromeric copy SMN2 which is retained in all patients. The SMN1 and SMN2 genes are virtually identical differing functionally by a single nucleotide. This change affects the activity of a splicing enhancer in exon 7. As a result the majority of the transcript from SMN2 lacks exon 7. We have screened a library of compounds in order to identify ones that alter the splicing pattern of the SMN2 gene.

Using a semi-quantitative RT-PCR assay, we show that the compound aclarubicin increases the incorporation of exon 7 into the transcripts from the SMN2 gene in fibroblasts derived from type I SMA patients. This effect is time- and dosage-dependent and it lasts up to 48 hrs from the withdrawal of the drug. Western blot and immunofluorescence analysis demonstrate that SMN protein levels and localization patterns are restored to normal in type I SMA cells after treatment with aclarubicin. We also show that this compound does not alter the splicing pattern of other genes and that this effect is not caused by a stress response on the cells. Serine/arginine-rich (SR) proteins play an important role in constitutive and alternative pre-mRNA splicing. The activity of these proteins is regulated by phosphorylation and we are currently investigating whether the effect of aclarubicin on SMN2 splicing is related to modification of the phosphorylation status of some SR proteins.

To our knowledge no drugs that modulate RNA splicing patterns have been previously reported. Our results suggest that compounds that alter specific splicing patterns of genes may represent a new group of targets for the development of drugs in disease therapy.

**Evaluation of pharmacological therapy in the ALD mouse: reduction of VLCFA levels without increased VLCFA degradation.** *M.C. McGuinness, J.-F. Lu, H.-P. Zhang, K.D. Smith.* Department of Neurogenetics, Kennedy Krieger Institute, Baltimore, MD.

X-linked adrenoleukodystrophy (XALD) is an inherited neurometabolic disease associated with elevated levels of saturated very long chain fatty acids (VLCFA: C>22:0) in plasma and tissues, and reduced peroxisomal VLCFA  $\beta$ -oxidation in fibroblasts, white blood cells and amniocytes from XALD patients. The XALD gene at Xq28 encodes a protein that is related to the ATP-binding cassette (ABC) transmembrane half-transporter proteins. The function of ALDP is unknown and its role in VLCFA accumulation unresolved. In our ALD mouse model, fibroblasts and tissue VLCFA levels are elevated. ALD mouse fibroblast VLCFA  $\beta$ -oxidation activity is reduced, as expected, but ALD mouse tissue  $\beta$ -oxidation activity is normal suggesting that ALDP is not required for VLCFA  $\beta$ -oxidation. We show that: **1)** 4-phenylbutyrate (4PBA), **2)** a structural analog of 4PBA, styrylacetate (SAA), and **3)** a functional analog of 4PBA, trichostatin A (TSA) treatment results in a decrease in VLCFA levels and an increase in VLCFA  $\beta$ -oxidation activity in human and mouse fibroblasts *in vitro*. In addition, we investigate the effect of these drugs *in vivo*: **1)** 4PBA (0.16g/kg/day), **2)** SAA (2.8g/kg/day), and **3)** TSA (3.04mg/kg/day and 0.30mg/kg/day) on both VLCFA accumulation and  $\beta$ -oxidation in ALD mouse liver, brain and adrenals. These pharmacological agents were tolerated well by the mice for up to 20 weeks. We show that each of these treatment regimens results in a decrease in VLCFA levels but has no effect on VLCFA  $\beta$ -oxidation activity providing further evidence that ALDP is not directly involved in VLCFA  $\beta$ -oxidation and that the levels of VLCFA are not determined by the rate of VLCFA  $\beta$ -oxidation. These studies contribute to a better understanding of the role of ALDP in VLCFA accumulation, and may lead to the development of more effective pharmacological therapies for XALD.

**Idebenone reduce cardiac hypertrophy in Friedreich ataxia.** *P. Rustin<sup>1</sup>, A. Munnich<sup>1</sup>, A. Rotig<sup>1</sup>, D. Sidi<sup>2</sup>.* 1) INSERM U-393, Hopital Necker Enfants Malades, Paris, France; 2) Service de Cardiologie Pdiatrique, Service de Cardiologie, Hpital Necker-Enfants Malades, Paris, France.

Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disease causing limb and gait ataxia and cardiomyopathy. The disease gene encodes a protein of unknown function, frataxin. The loss of frataxin is due to a large GAA trinucleotide expansion in the first intron of the gene and causes an oxidative stress with a combined deficiency of a Krebs cycle enzyme, aconitase, and three mitochondrial respiratory chain complexes (I-III). Idebenone, a short-chain quinone acting as an antioxidant, has been originally shown to protect heart muscle against oxidative stress (Rustin et al, 1999, Lancet 354:477-9). We have carried out a study in a series of FRDA patients aiming to determine the factors that potentially control the effect of the drug on the left ventricular mass and function. Idebenone (5 mg/kg/day) was given orally to 38 FRDA patients aged 4-22 years (20 boys, 18 girls). Heart ultrasound parameters were recorded on the same scanner prior to and after idebenone treatment. After six months, heart ultrasound indicated a more than 20% reduction of left ventricular mass in about 50% of patients ( $p < 0.001$ ). The shortening fraction first decreased in 6/38 subjects (11-26%) and then improved in 5/6 individuals. In one patient, the shortening fraction only responded to 10 mg/kg/day of idebenone. No correlation between responsiveness to idebenone and age, sex, initial ultrasound parameters or number of GAA repeats in the frataxin gene was found. These data indicate that none of the parameters studied has a predictive value for the effectiveness of the treatment. This suggests that idebenone is worth trying in FRDA patients irrespective of the size of the GAA expansion or the initial ultrasound parameters.

**SMN2 gene stimulations give differential expression in SMA and control cultured cells.** *P. Burlet, E. Vial, L. Violette, C. Huber, A. Munnich, S. Lefebvre.* INSERM U393, IFREM, Hopital Necker Enfant, Paris, France.

The Survival Motor Neuron (SMN1) gene is absent in the majority of patients with Spinal Muscular Atrophy (SMA) and a highly homologous copy (SMN2) is still present. We showed a tight correlation between the SMN2 protein level and the clinical severity, suggesting that SMN2 acts as a major modifier gene. There is no cure for this devastating disease and an attractive therapeutic strategy could be to up-regulate SMN2 gene expression. First, a computer search allowed us to identify several binding sites for transcription factors in the nearly identical SMN1 and SMN2 promoters. By immunoblot analysis, two compounds out of eight triggered a two-fold increase of the SMN protein level in fibroblast and lymphoblastoid cell lines from SMA patients and control individuals. The effects on the SMN protein were further investigated by immunocytochemistry analyses and subcellular fractionation experiments. The SMN protein is located both in the nucleus and in the cytoplasm. Interestingly, one molecule increased the cytoplasmic pool of SMN while the other acted on the nuclear pool. The role of the different subcellular fractions of the SMN protein and which altered compartment is responsible for SMA is still poorly understood. The current study should help at identifying which SMN pool is the most relevant when screening pharmacological molecules in order to find a treatment for SMA.

**System analysis of the energies of the triple base triplets.** *S.Q. Liu<sup>1</sup>, J. Yang<sup>1</sup>, C.L. Bai<sup>2</sup>, C.Q. Liu<sup>1</sup>*. 1) Laboratory of cellular and molecular evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming YN, 650223, P. R. China; 2) Chemistry Institute, Chinese Academy of Science, Beijing, 100081, P. R. China.

The oligodeoxyribonucleic acid (ODN) directed triple helix formation could be used to control transcription, DNA replication, and recombination, it has widely been applied in therapeutics, diagnostics and antigene strategy. TBT is a kind of structural constituent of triplex DNA. It contains three deoxyribonucleic acid strands; each of them comprises three bases (a codon). The TBT can be observed as three layers of base triplets. 64 sets of 3D models of DNA triplex base triplets were built by homologous modeling method. According to the nucleotide sequence of TBT is symmetric or asymmetric, and the orientations of the third ODN strand binding to target duplex DNA in parallel or antiparallel direction, the 4 groups of energy data of monomers and water-K<sup>+</sup>-TBT ternary complexes of TBT were calculated and analyzed. The results showed: i. the energies of the symmetric parallel ternary complexes of TBT are higher than those of the symmetric antiparallel ternary complexes of TBT; ii. No matter TBTs are monomers or ternary complexes, the energies of asymmetrical parallel TBTs are generally lower than those of the asymmetrical antiparallel TBTs; iii. The duplex DNA of which any strand contains at least a couple of A or T has a preference for selecting the ODN strand containing abundant T to form TBT. iv. The duplex of which any strand contains at least a couple of G or C has a preference for selecting ODN containing abundant G to form symmetric antiparallel TBT, but selecting ODN containing abundant C to form asymmetric parallel TBT. v. The duplex of which any strand contains only one of A, T, G or C has a preference for selecting ODN containing at least a couple of pyrimidines to form antiparallel TBT. our result suggests the sequences of the TBTs and the orientations of the third ODN strands are two of key factors that can influence formation and stability of TBT, it provides directive significance for designing ODN strand in the fields of therapeutics and antigene drug design.



**Construction and Characterization of a replication competent Retroviral Shuttle Vector.** *J. Oh, S.H. Hughes.* HIV Drug Resistance Program, NCI at Frederick, Frederick, MD.

We constructed a RCAS-based retroviral shuttle vector, RSVP (RCAS Shuttle Vector Plasmid), containing either the zeocin or blasticidin resistance gene. In this vector, the drug resistance gene is expressed in avian cells from the LTR promoter, whereas in bacteria the same gene is expressed from a bacterial promoter. The vector contains a bacterial origin of replication (ColE1) to allow replication in bacteria. The vector also contains the lac operator sequence, which binds to the lac repressor protein allowing simple and rapid purification of the vector DNA. Plasmid RSVP, therefore, contains the following sequence: 5'(LTR, gag, pol, env, drug resistance gene, lac operator, ColE1, LTR) 3'. After this vector was transfected into DF-1 cells, we were able to rescue the circularized unintegrated viral DNA from RSVP simply by transforming the HIRT DNA into *E. coli* cells. Furthermore, we were able to rescue the integrated viral DNA. DNA from infected cells was digested with an appropriate restriction enzyme (ClaI) and the vector containing segments were enriched using lac repressor protein and then self ligated. These enriched fractions were used to transform *E. coli*. The vector is relatively stable upon passage in avian cells. Southern Blot analyses of genomic DNAs derived from several viral passages under selective or nonselective conditions showed that the cassette (drug resistance gene-lac operator-ColE1) insert was present in the vector up to third viral passage for both drugs, which suggests that the RSVP vector is stable for approximately 3 viral passages. Together, these results showed that RSVP vector is useful tool for cloning unintegrated or integrated viral DNAs.

**Adenoviral Vector Systems for Delivery of Dystrophin cDNA Expression Constructs to Mouse Skeletal Muscle.**

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Gene therapy using viral vectors holds promise for several human genetic diseases but much remains to be learned about which vector system will meet all of the requirements of such a treatment. Our laboratory has focused on modification of adenoviral (Ad) vectors in order to improve the efficiency of delivering expression constructs to skeletal muscle with respect to transgene expression levels, persistence, immune response, and tissue-specific vs. constitutive promoters. Improvements in first generation Ad vectors that carry mini-dystrophin cassettes was achieved by deleting the viral polymerase gene which rendered the vector replication defective and eliminated viral late gene expression in transduced cells. We have also made gutted Ad vectors lacking all viral ORFs, and adeno-associated virus (AAV) containing a micro-dystrophin expression cassette. These systems are compared using the *mdx* mouse model of Duchenne muscular dystrophy whereby skeletal muscle is injected and assays are performed to evaluate dystrophin protein expression levels, persistence of the transgene, immune cell infiltration, and integrity of the muscle fiber membranes. Our results indicate that gutted Ad vectors containing a dystrophin expression cassette driven by a strong muscle-specific promoter (modified MCK) lead to significantly increased transgene expression and decreased immune response compared to the first generation viruses. We find that the MCK promoter is highly effective in reducing the antigen-specific immune response against Ad vectors despite the muscle cell death and antigen-presenting cell infiltration in dystrophic muscle. A gutted Ad vector expressing full-length utrophin is also being compared with the dystrophin vector for function, persistence and immunogenicity. Additional improvements have been noted using an AAV vector which exhibits robust expression of dystrophin and even less immune response than that elicited by Ad vectors. These results show that modified Ad and AAV vectors can lead to significant and prolonged gene expression in dystrophic muscle.